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FEE TRANSMITTAL
For FY 2008

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 540.00

Complete if Known	
Application Number	10/018,373
Filing Date	December 6, 2001
First Named Inventor	Hans BIGALKE
Examiner Name	Vanessa L. FORD
Art Unit	1645
Attorney Docket No.	MERZ 32 PCT US

OIPE IAP2B OCT 07 2008 PATENT & TRADEMARK OFFICE

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FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Application Type	FILING FEES Fee ($)	FILING FEES Small Entity Fee ($)	SEARCH FEES Fee ($)	SEARCH FEES Small Entity Fee ($)	EXAMINATION FEES Fee ($)	EXAMINATION FEES Small Entity Fee ($)	Fees Paid ($)
Utility	310	155	510	255	210	105	
Design	210	105	100	50	130	65	
Plant	210	105	310	155	160	80	
Reissue	310	155	510	255	620	310	
Provisional	210	105	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	210	105
Multiple dependent claims	370	185

Total Claims ____ - 20 or HP = **Extra Claims** ____ x **Fee ($)** ____ = **Fee Paid ($)** ____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims ____ - 3 or HP = **Extra Claims** ____ x **Fee ($)** ____ = **Fee Paid ($)** ____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims **Fee ($)** ____ **Fee Paid ($)** ____

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $260 ($130 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets ____ - 100 = **Extra Sheets** ____ / 50 = **Number of each additional 50 or fraction thereof** ____ (round **up** to a whole number) x **Fee ($)** ____ = **Fee Paid ($)** ____

4. OTHER FEE(S) **Fees Paid ($)**

Non-English Specification, $130 fee (no small entity discount) ____

Other (e.g., late filing surcharge): Filing a Brief in Support of an Appeal under 37 CFR § 41.20(b)(2) 540.00

SUBMITTED BY

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Name (Print/Type)	G. PATRICK SAGE		Date October 7, 2008

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:	Hans BIGALKE and Jürgen FREVERT
Serial No.:	10/018,373
Filed:	December 6, 2001
For:	Therapeutic Composition Comprising a Botulinum Neurotoxin
Art Unit:	1645
Examiner:	Vanessa L. FORD

APPEAL BRIEF ON BEHALF OF APPELLANT UNDER 37 CFR § 41.37

TABLE OF CONTENTS

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Mail Stop: Appeal Brief
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

INTRODUCTORY COMMENTS

Further to the Notice of Appeal filed on August 15, 2008, Appellant hereby requests reconsideration and reversal of the rejection of Claims 11-18, which claims are finally rejected in the Final Office Action dated June 2, 2008.

I. REAL PARTY IN INTEREST

As evidenced by the Assignment executed November 19, 2001, recorded at reel 012742, frame 0041, the Real Party in Interest in the present application is the Assignee of record, MERZ PHARMA GMBH & CO. KGAA of Frankfurt, Germany.

II. RELATED APPEALS, INTERFERENCES AND JUDICIAL PROCEEDINGS

There are no pending appeals or interferences relating to the present application known to Appellant or Appellant's Legal Representatives.

III. STATUS OF CLAIMS

Claims 11-18 are pending. Claims 11-18 were rejected in the FINAL Office Action dated June 2, 2008; the Office Action dated November 5, 2007; the Final Office Action dated May 3, 2007; the Non-Final Office Action dated August 1, 2006, the Office Action dated January 25, 2005; the Office Action dated July 9, 2004 and were rejected in the Non-Final Office Action dated January 13, 2004. As a result, pending Claims 11-18 are presented for appeal. The claims do not stand or fall together, but are grouped as discussed further below.

IV. STATUS OF AMENDMENTS

All amendments presented by Appellant have been entered. No amendments were filed subsequent to the Office Action dated November 5, 2007, therefore, the status of the claims is as displayed in the claims appendix attached hereto.

V. SUMMARY OF CLAIMED SUBJECT MATTER ON APPEAL

Two independent claims are presented on appeal, namely Claim 11 and Claim 16. The instant specification describes the invention; specific disclosure relating to the individual claim elements is identified.

Independent Claim 11 is directed to a method of treating a human or animal with a cosmetic condition treatable with a botulinum neurotoxin (page 8, second paragraph), comprising administering a treatment effective amount of a botulinum neurotoxin from *Clostridium botulinum* of type A, B, C1, D, E, F or G or a mixture of two or more botulinum neurotoxins (page 1, second paragraph and continuing to page 2), wherein the neurotoxin or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum neurotoxins, (page 2, second paragraph) and wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes (page 4, first full paragraph; page 5, last paragraph, continuing at page 6; page 8, third paragraph; Example 7 at page 15 and Example 8 at page 16).

Independent Claim 16 is directed to a method of treating a human or animal with dystonia or a nervous system disorder treatable with a botulinum neurotoxin (page 8, first paragraph), comprising administering a treatment effective amount of a botulinum neurotoxin from *Clostridium botulinum* of type A, B, C1, D, E, F or G or a mixture of two or more botulinum neurotoxins, wherein the neurotoxin or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum neurotoxins, and wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes. (see for example, the pages and paragraphs noted above, as well as page 8, first paragraph).

Both of Claims 11 and 16 are characterized by being a method of treating a human or animal with a condition treatable with a botulinum neurotoxin, comprising administering a treatment effective amount of a botulinum neurotoxin from *Clostridium botulinum* of type A, B, C1, D, E, F or G or a mixture of two or more botulinum neurotoxins, wherein the neurotoxin or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum

neurotoxins, and wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes; the claims are distinguished for the fact that Claim 11 is directed to a method of treating cosmetic conditions and Claim 16 is directed to a method of treating dystonia or a nervous system disorder treatable with a botulinum neurotoxin.

The methods of the present invention are applicable for the treatment of *inter alia* various neuromuscular disorders in subjects who already exhibit neutralizing antibodies against botulinum neurotoxin complexes, including blepharospasm, hemifacial spasms, spasmodic torticollis, spasticities, migraine, low back pain, cervical spine disorders, hypersalivation, and dystonias, as well as for cosmetic conditions, such as pronounced wrinkling and hyperhidrosis (see for example page 1, second paragraph, continuing to page 3 and page 8, first and second paragraphs).

Appellant explains that conventional *Clostridium botulinum* toxin preparations, such as BOTOX® and DYSPORT®, comprise toxin complexes for the treatment of various dystonias, disorders of the nervous system and cosmetic conditions (see page 1, second paragraph). Furthermore, Appellant explains that subjects treated with conventional toxin complex preparations develop neutralizing antibodies directed against the neurotoxin upon repeated administration of the botulinum toxin complexes (see page 3, first paragraph). Appellant explains that, as a direct consequence of antibody development, antibody-positive patients no longer respond to the complex, and therefore, further therapy is discontinued. (see page 3, first paragraph).

Appellant has found that botulinum neurotoxins which are free from complexing proteins which naturally form complexes with botulinum neurotoxins, exhibit unexpected efficacy in subjects who exhibit neutralizing antibodies to botulinum toxin complexes (see Example 7 at page 15 and Example 8 at page

16). With the instant invention, Appellant has provided a solution to a an unsolved need which has long existed in the art and resulted in the futility of the treatment of subjects who already exhibit neutralizing antibodies to botulinum toxins and who are in need of *Clostridium botulinum* neurotoxin therapy.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

There are four remaining grounds of rejection in this application which are articulated in the Final Office Action dated June 2, 2008, and those rejections are as follows:

(A) whether Claims 11 and 14-15 are unpatentable under 35 U.S.C. § 103(a) over *Keen, et al.* (Plastic and Reconstructive Surgery, July 1994, 94:94-99) in view of *Johnson, et al.* (U.S. Patent No. 5,512,547 published April 30, 1996).

(B) whether Claims 11-15 are unpatentable under 35 U.S.C. § 103(a) over *Carruthers, et al.* (Cosmetic Uses of Botulinum Toxin A Exotoxin, In: Klein AW, ed. *Tissue Augmentation in Clinical Practice: Procedures and Techniques*. New York: Marcel Dekker, 1998, p. 207-236) in view of *Heckman, et al.* (Arch. Dermatol., 1998, 134:1298-1299) and further in view of *Johnson, et al.* (U.S. Patent No. 5,512,547, published April 30, 1996).

(C) whether Claims 16-18 are unpatentable under 35 U.S.C. § 103(a) over *Kessler, et al.* (J. Neurol., 1999, 246:265-274) in view of *Johnson, et al.* (U.S. Patent No. 5,512,547, published April 30, 1996).

(D) whether Claims 16-18 are unpatentable under 35 U.S.C. § 103(a) over *Göschel, et al.*, (Experimental Neurology 1997, 147:96-102) in view of *Johnson, et al.* (U.S. Patent No. 5,512,547, published April 30, 1996).

VII. APPELLANT'S ARGUMENTS

Impropriety of first grounds of rejection:

(A) Rejection of Claims 11 and 14-15 under 35 U.S.C. § 103(a) over *Keen, et al.* (Plastic and Reconstructive Surgery, July 1994, 94:94-99) in view of *Johnson, et al.* (U.S. Patent No. 5,512,547 published April 30, 1996).

It is well-settled that to establish *prima facie* obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

Appellant's claimed invention is a method of treating a human or animal with a botulinum neurotoxin from *Clostridium botulinum* of type A, B, C, D, E, F or G or a mixture of two or more neurotoxins, wherein the neurotoxin is free of the complexing proteins which naturally form complexes with botulinum neurotoxins, and wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes.

1. The Examiner has not established a proper case of *prima facie* obviousness; the Examiner has not established that all claim limitations are taught or suggested by the prior art.

1a. The claim limitation to a method of treating a human or animal with a cosmetic condition treatable with a botulinum neurotoxin, wherein the neurotoxin is free of complexing proteins which

naturally form complexes with botulinum neurotoxins, and "wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes" is not taught or suggested by the prior art.

The cited art do not disclose or suggest the instant generic claim limitation, "wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes" in view of the teaching of the cited art. (see Appellant's Response of July 27, 2005 at page 7 and Appellant's Response of January 3, 2008 at page 3).

The *Johnson, et al.* disclosure pertains to shelf-stable botulinum neurotoxin preparations for preventing the development of neutralizing antibodies. *Johnson, et al.* disclose that pharmaceutical compositions made from a liquid formulation containing essentially pure botulinum type A neurotoxin, albumin and trehalose provides for the improved recovery of active toxin following lyophilization. *Johnson, et al.* state that, "This improvement also reduces the amount of inactive toxin (toxoid) in each vial and thereby lessens the possibility of antibody formation after injection of the preparation into patients." (see Column 2, lines 47-51). The Examiner acknowledges that *Johnson, et al.* teach that, "higher specific activity preparations reduce the *probability of patients developing* neutralizing antibodies". (see the November 5, 2007 Office Action at page 4, *emphasis added*). Moreover, *Johnson, et al.* teach that, "One way to reduce the number of patients developing neutralizing antibodies would be to develop a more shelf-stable product with a higher specific activity following lyophilization. Such a formulation would result in a product that is not as antigenic as the currently available product and lesser quantities of toxin would be required for treatment." (see Johnson, et al. at Column 1, lines 55-61). Therefore, *Johnson, et al.* teach botulinum neurotoxin preparations for reducing the possibility of antibody formation when administered to a patient, which

teaching is acknowledged by the Examiner (see the Office Action of June 2, 2008 at pages 5-6).

The Examiner states on the record that *Keen, et al.* do not teach the claim limitation "wherein the neurotoxins or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum neurotoxins and wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes". (see the Office Action of November 5, 2007 at page 4). The Examiner attempts to cure this deficiency of disclosure by citing *Johnson, et al.* for teaching of purified (without complexing proteins) botulinum toxin compositions and states on the record that, "*Johnson, et al.* teach that a purified product reduces the amount of inactive toxin in each vial and thereby lessens the possibility of antibody formation after injection of the preparation into patients". (see the Office Action of November 5, 2007 at page 5 and the Office Action of June 2, 2008 at pages 5-6). Appellant does not dispute that *Johnson, et al.* teach a method of reducing antibody formation in a patient, which teaching has been discussed in Appellant's response to the previous rejection based on *Keen, et al.* and *Johnson, et al.* (see Appellant's Response of January 3, 2008 at page 3).

Significantly and critically, *Keen, et al.* and *Johnson, et al.* do not teach or suggest the instant claim limitation to administration of a *Clostridium botulinum* neurotoxin which is free of complexing proteins to subjects already exhibiting neutralizing antibodies. In fact, the *Keen, et al.* and *Johnson, et al.* disclosures teach that subjects who have developed neutralizing antibodies would not benefit from treatment with botulinum neurotoxins. *Keen, et al.* state that, "The antibodies can render the toxin ineffective but do not harm the patient." (see page 98). *Johnson, et al.* state that, "The toxin is recognized by patient's immune systems as foreign and stimulates antibody production. This renders treatment of the various hyperactive muscle disorders with botulinum toxin ineffective." (see

Column 1, lines 51-55). Appellant submits that the cited art teach that administering *Clostridium botulinum* neurotoxin to patients who have developed neutralizing antibodies is futile, and therefore, teach away from administering botulinum neurotoxin to subjects already exhibiting neutralizing antibodies against botulinum neurotoxin complexes.

The Examiner acknowledges that *Keen, et al.* do not teach the claim limitation, "wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes". (see the November 5, 2007 Office Action at page 4).

The fact that *Johnson, et al.* <u>do not</u> disclose or teach administration of a botulinum neurotoxin preparation which is free from complexing proteins to subjects <u>already exhibiting neutralizing antibodies</u> was discussed during the January 3, 2008 interview with the Examiner and her supervisor, as well as in Appellant's Response of January 3, 2008 at page 3. Appellant pointed out that *Johnson, et al.* administer various *Clostridium botulinum* neurotoxin formulations to <u>naïve</u> animals to assess the development of antibodies and conclude that no neutralizing antibodies are formed in the subject animals when shelf-stable formulations of the invention are administered (see *Johnson, et al.*, Columns 5-7 and Table 3) to substantiate the understanding that *Johnson, et al.* do not disclose or teach administration of a botulinum neurotoxin in a subject who already exhibits neutralizing antibodies directed against neurotoxin complexes.

Appellant's statements and evidence on the record of the instant application demonstrate that *Keen, et al.* and *Johnson, et al.* do not disclose the critical claim limitation, "wherein the human or animal <u>already exhibits neutralizing antibodies against botulinum neurotoxin complexes</u>". The criticality of such limitation is the subject of Appellant's application, an advance in the treatment of patients who are non-responsive to conventional botulinum

neurotoxin therapy and which treatment meets the unsolved needs of patients exhibiting neutralizing antibodies and who have been heretofore untreatable with botulinum neurotoxin therapy. Therefore, the Examiner's rejection should be reversed for failing to demonstrate all claim limitations to be taught or suggested by the art. Absent such showing, the Examiner has not met her burden to demonstrate that the rejected claims are *prima facie* obvious after repeated solicitations from Appellant.

2. The Examiner has not established a proper rejection for *prima facie* obviousness by identifying a motivation to combine the teaching of the prior art references.

It is well-settled that a *prima facie* rejection for obviousness is only established if there is some suggestion or motivation to combine the prior art references, and the teaching or suggestion to make the combination, together with a reasonable expectation of success, must both be found in the prior art, not based on Applicant's own disclosure. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991). This requirement has been established to prevent the use of improper hindsight combinations of prior art.

As set forth above, the cited art teaches away from administering botulinum neurotoxin to subjects already exhibiting neutralizing antibodies against botulinum neurotoxin complexes. Furthermore, there is nothing in the cited art that would provide one skilled in the art with any motivation to administer botulinum neurotoxin with a reasonable expectation of successfully treating a subject who already exhibits neutralizing antibodies.

The Examiner cites *Keen, et al.* for teaching a method of treating patients that have hyperkinetic facial lines (wrinkles) with injections of botulinum toxin A

complex. The Examiner states on the record that, "*Keen, et al.* teach that antibodies to botulinum toxin A have been described in patients receiving much larger dosages of botulinum toxin complex for long periods of time and the antibodies can render the toxin non-effective but do not harm the patient (nonresponders)." The Examiner states on the record, however, that *Keen, et al.* do not teach the claim limitation, "wherein the neurotoxins or mixture of neurotoxins is free of the complexing protein which naturally form complexes with botulinum neurotoxins and <u>wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes</u>." (see the Office Action of November 5, 2007 at pages 3-4 and the Office Action of June 2, 2008 at page 5).

The Examiner cites *Johnson, et al.* for teaching a composition comprising an essentially pure botulinum toxin A (free from complexing proteins). Furthermore, the Examiner states on the record that, "*Johnson, et al.* teach the compositions of the invention reduces the amount of inactive toxin in each vial and thereby lessens the possibility of antibody formation after injection of the composition into patients." (see the Office Action of June 2, 2008 at pages 5-6). Moreover, the Examiner states that, "*Johnson, et al.* teach that higher specific activity toxin preparations reduce the probability of patients developing neutralizing antibodies, and it would be obviously desirable to have higher specific activity preparations than those currently available." (see the Office Action of November 5, 2007 at pages 4-5).

The Examiner's basis for finding the instant invention *prima facie* obvious is that it would have been obvious at the time of the invention to substitute botulinum toxin A complex in the method of treating patients with hyperkinetic facial lines (wrinkles) as taught by *Keen, et al.* with the pure botulinum toxin A (without complexing proteins) as taught by *Johnson, et al.* because *Johnson, et al.* teach that purified product reduces the amount of inactive toxin in each vial

and thereby *lessens the possibility of antibody formation* after injection of the preparation into patients. (see the Office Action of November 5, 2007 at page 5, *emphasis added*).

In fact, the instant enquiry is whether, based on *Johnson, et al.* and *Keen, et al.*, it would have been obvious to administer a botulinum neurotoxin which is free from complexing proteins which naturally form complexes with botulinum neurotoxins to patients already exhibiting neutralizing antibodies. This is the subject matter of the claims, not whether a composition may be expected to reduce the possibility of developing neutralizing antibodies.

Appellant submits that the teaching of a method reducing the probability of antibody formation in a patient, as taught in *Johnson, et al.*, does not provide a motive or expectation of success for one skilled in the art to modify the disclosure of *Keen, et al.* and administer a botulinum neurotoxin to a subject who already exhibits neutralizing antibodies. Appellant has already discussed that *Keen, et al.* and *Johnson, et al.* do not teach treating a subject who already exhibits neutralizing antibodies to botulinum neurotoxin complexes. Moreover, as discussed above, *Keen, et al.* and *Johnson, et al.* teach that such administration to subjects already exhibiting neutralizing antibodies would be ineffective (see *Johnson, et al.* at Column 1, lines 51-55 and *Keen, et al.* at page 98, right column).

The fact that "*Johnson, et al.* teach that, "the compositions of the invention reduces the amount of inactive toxin in each vial and thereby *lessens the possibility of antibody formation* after injection of the composition into patients" (Quote, see the Office Action of June 2, 2008 at pages 5-6, *emphasis added*) and that, "*Johnson, et al.* recognize patients that have developed neutralizing antibodies to the complex are a growing concern in the art, thus this is the very bases for the development of compositions comprising essentially pure botulinum

toxin." (Quote, see the Office Action of June 2, 2008 at pages 6-7, *emphasis added*) is laudable, but not relevant to the instant inquiry.

The Examiner must establish that one skilled in the art actually has taught, or may infer from the combined disclosure of record, any teaching or suggestion of administering a botulinum neurotoxin, wherein the neurotoxin is free of complexing proteins which naturally form complexes with botulinum neurotoxins, to a human or animal subject <u>already exhibiting neutralizing antibodies</u> to botulinum neurotoxin complexes. Such a showing is not on the record in the several iterations of this rejection.

The Examiner does not point to any teaching in *Johnson, et al.* or *Keen, et al.* suggesting that it would be suitable or desirable to provide the disclosed compositions to a subject who already exhibits neutralizing antibodies. Moreover, the Examiner has not explained where or why *Johnson, et al.* would have suggested to one of ordinary skill that it would be desirable to administer a purified botulinum neurotoxin to a subject who already exhibits neutralizing antibodies. In fact, the Examiner has arbitrarily and prejudicially ignored Appellant's repeated citation of the *Johnson, et al.* and *Keen, et al.* teaching that the presence of neutralizing antibodies in a patient renders treatment with botulinum toxin ineffective.

Regarding the teaching of *Johnson, et al.*, the Examiner's stated position that, "Therefore, it would be obvious to administer these compositions to patients that have developed neutralizing antibodies to the botulinum toxin complex." finds no basis in the prior art of record. Absent specific citation in the art, the Examiner's statements are either inappropriate opinion or speculation.

The mere fact that references can be combined or modified does not render the resulting combination obvious unless the results would have been predictable

to one skilled in the art. *KSR International Co. v. Teleflex Inc., 127 S. Ct. 1727, 1740-41, 82 USPQ2d 1385, 1396 (2007).* The Court recognized that it is often necessary to look at the interrelated teachings of multiple references in order to determine whether there is an apparent reason to combine the known elements in the fashion claimed. *Id. at 1740-41*

What is more, the cited references do not, in fact, comprise the requisite teaching. *Johnson, et al.* teach away from administering botulinum neurotoxin to a subjects already exhibiting neutralizing antibodies. *Johnson, et al.* state that, "A major drawback to the use of botulinum toxin in treatment of hyperactive muscle disorders is development of antibodies or other types of immunity by patients. The toxin is recognized by patient's immune systems as foreign and stimulates antibody production. This renders treatment of the various hyperactive muscle disorders with botulinum toxin *ineffective.*" (see *Johnson, et al.*, Column 1, lines 49-55, *emphasis added).* The Examiner acknowledges that, "*Keen, et al.* teach that patients that receive large dosages of botulinum toxin complex over long periods of time can render the toxin non-effective (e.g. these patients are non-responders)." (see the Office Action of June 3, 2008 at page 5). Moreover, the Examiner acknowledges that *Keen, et al.* teach that, "the antibodies can render the toxin *noneffective.*" (see the November 5, 2007 Office Action at page 4).

Appellant submits that *Keen, et al.* and *Johnson, et al.* teach that administration of a botulinum neurotoxin to a subject who <u>already exhibits neutralizing antibodies</u> is futile, thereby teaching away from the instant claims to administering botulinum neurotoxin to a patient already exhibiting neutralizing antibodies against neurotoxin complexes.

Moreover, the Examiner articulates further bases for rejecting the claims for obviousness over the disclosure of *Keen, et al.* and *Johnson, et al.*, stating that

"One of ordinary skill in the art would reasonably conclude that the compositions taught by *Johnson, et al.* comprise botulinum toxin formulations that <u>lessen or reduce neutralizing antibodies</u> because it contains essentially pure botulinum toxin. *Johnson, et al.* recognize patients that have developed neutralizing antibodies to the complex are a growing concern in the art, thus this is the very bases for the development of compositions comprising essentially pure botulinum toxin." Based on this reasoning, the Examiner concludes that, "Therefore, it would be obvious to administer these compositions to patients that have developed neutralizing antibodies to the botulinum toxin complex". (see the Office Action of June 2, 2008 at page 6).

Appellant submits that the *Johnson, et al.* teaching of administering a purified botulinum neurotoxin to prevent antibody formation provides no correlation to treating subjects who already exhibit neutralizing antibodies. Appellant submits that the Examiner has not articulated an adequate rationale for the conclusion that, "Therefore, it would be obvious to administer these compositions to patients that have developed neutralizing antibodies to the botulinum toxin complex." The Examiner has not provided any basis in fact or technical reasoning as to why it would be obvious to administer a botulinum neurotoxin to a subject who <u>already exhibits neutralizing antibodies</u> based on the teaching of <u>preventing the development</u> of neutralizing antibodies in naïve subjects, as taught in *Johnson, et al.*

Because the Examiner did not explain the specific understanding or principle within the knowledge of a skilled artisan that would motivate one with no knowledge of Appellant's invention to allege that it would be obvious to administer botulinum neurotoxin which is free of complexing proteins to a subject already exhibiting neutralizing antibodies to neurotoxin complexes, Appellant submits that the Examiner's rejection is without basis.

Furthermore, the Examiner concludes that, "It would be expected absent, evidence to the contrary, that a composition comprising pure botulinum toxin A (without complexing proteins) would be effective in treating patients that are nonresponders (have neutralizing antibodies to botulinum toxin A complex) because *Johnson, et al.* teach that higher specific activity preparations reduce the probability of patients developing neutralizing antibodies and it would be obviously desirable to have higher specific activity preparations than those currently available." (see the Office Action of November 5, 2007 at page 5).

Appellant submits that the Examiner has not fulfilled the obligations on the Office to establish a *prima facie* basis for alleging obviousness. It is well-established that the Office has the initial burden of establishing and factually and rationally supporting the conclusion that the claims are *prima facie* obvious; the Examiner's basis for obviousness cannot be sustained by mere conclusory statements.

Appellant submits that the factual information in the art of record regarding the ineffectiveness of botulinum neurotoxin treatment in subjects with neutralizing antibodies against botulinum neurotoxin complexes, as discussed above, demonstrates that the Examiner's stated position that, "It would be expected absent, evidence to the contrary, that a composition comprising pure botulinum toxin A (without complexing proteins) would be effective in treating patients that are nonresponders (have neutralizing antibodies to botulinum toxin A complex)" finds no basis in the prior art disclosure of record.

3. The Examiner misapplied and fails to substantiate a finding of obviousness under *KSR International Co. v. Teleflex Inc.*

The Examiner recites exemplary rationales to support a finding of obviousness. The Examiner states that "Additionally, *KSR International Co. v.*

Teleflex Inc., 127 S. Ct. 1727, 1741 (2007) discloses that if a technique has been used to improve one method and a person of ordinary skill would recognize that it would be used in similar methods in the same way, using the technique is obvious unless its application is beyond that person's skill." (see the June 2, 2008 Office Action at page 6). Furthermore, the Examiner states that, "*KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) also discloses that the combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." (see the June 2, 2008 Office Action at page 6).

Appellant rebuts the Examiner's conclusions that the instant invention is obvious based on the Examiner's limited reasoning. According to MPEP § 2143 interpreting *KSR*, the Examiner must demonstrate that the prior art disclose a "comparable" method which has been <u>improved in the same way as that method of the claimed invention</u>.

The Examiner states on the record that, "*Johnson, et al.* also recognized that there is a need in the art to solve the problem of the *development of neutralizing antibodies* to the botulinum toxin complex. *Johnson, et al.* provided a solution to this problem, by preparing a product that is a pure neurotoxin instead of the complex." (Quote, pages 6-7 of the June 2, 2008 Office Action, *emphasis added*). The improvement method of the cited art, according to the Examiner's reasoning is <u>prevention of the development of neutralizing antibodies</u>; which method has already been demonstrated on the record to be a method which is not comparable to the instant method of treating subjects who <u>already exhibit neutralizing antibodies</u> to botulinum neurotoxin complexes. (see Appellant's Response of January 3, 2008 at page 3).

The Examiner has not articulated reasoning within the KSR guidelines as to why it would be obvious to administer a botulinum neurotoxin to subjects <u>already</u>

exhibiting neutralizing antibodies based on the teaching of preventing the development of neutralizing antibodies in *Johnson, et al.* Appellant submits that the Examiner's basis for obviousness cannot be sustained by mere conclusory statements and speculation.

Furthermore, Appellant submits that the Examiner's second recitation of exemplary *KSR* bases for obviousness, namely that, "The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." is redundant on the last basis evaluated and no more substantiated. Absent demonstrated teaching that the improvement is a predictable use of prior art elements according to their established functions, Appellant rebuts the Examiner's rejection as being wholly without basis.

The third exemplary rationale recited by the Examiner as to why the claimed invention would have been obvious in view of *KSR* is that it would be, "obvious to try". (see the June 2, 2008 Office Action at page 7).

According to MPEP § 2143 interpretation of *KSR,* to reject the claims based on the "obvious to try" exemplary rationale to support a finding of obviousness, the Examiner must articulate a finding that there had been a finite number of identified, predictable potential solutions to the recognized need or problem and that one of ordinary skill in the art could have pursued the known potential solutions with a reasonable expectation of success. Moreover, it is incumbent upon the Examiner to make the factual determinations set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966).

Appellant submits that the Examiner's third recitation of exemplary *KSR* bases for obviousness, namely that the instant method of treating subjects who already exhibit neutralizing antibodies against neurotoxin complexes with

botulinum neurotoxin which is free of complexing proteins is "obvious to try", is not substantiated.

To begin, the Examiner has, in fact, identified NO predictable potential solutions to the problem of treating subjects who already exhibit neutralizing antibodies against neurotoxin complexes. As discussed above, the cited art teach that neutralizing antibodies directed against botulinum neurotoxin are the cause of therapeutic failure and that subjects who exhibit neutralizing antibodies would not benefit from treatment with botulinum neurotoxin, i.e., there are NO identified predictable potential solutions. Appellant has already discussed that *Keen, et al.* and *Johnson, et al.* teach that the presence of neutralizing antibodies directed against botulinum neurotoxin renders the toxin ineffective in subjects who exhibit neutralizing antibodies. The cited art teaching that neutralizing antibodies against botulinum neurotoxin complexes are the cause of therapeutic failure was also discussed in Appellant's previous responses. (see Appellant's Response of April 2, 2004 at page 4; Appellant's Response of July 27, 2007 at page 4; and Appellant's Response of January 3, 2008 at page 3).

The Examiner has, however, capriciously mischaracterized the cited disclosure, independently concluding that *Johnson, et al.* "solve the problem associated with patients that <u>*have developed*</u> neutralizing antibodies to botulinum toxin complex." (Quote, June 2, 2008 Office Action at page 8, *emphasis added*). The Examiner has not identified disclosure in *Johnson, et al.* in which *Johnson, et al.* solves, much less speaks to, problems associated with patients who <u>have already developed</u> neutralizing antibodies to botulinum toxin complex.

Appellant asserts that *Johnson, et al.*, as a whole, teaches that, "The toxin is recognized by patient's immune systems as foreign and stimulates antibody production. <u>This renders treatment of the various hyperactive muscle disorders with botulinum toxin ineffective</u>." (see Column 1, lines 51-55). Appellant's

method for treating subjects already exhibiting neutralizing antibodies would not have been obvious to one of ordinary skill in the art after consideration of the teaching of *Johnson, et al.*

Appellant submits that the teaching of the art of record does not provide a single predictable potential solution or any basis why one skilled in the art would have a reasonable expectation of success for treating a subject who already exhibits neutralizing antibodies against botulinum neurotoxin complexes with botulinum neurotoxin, wherein the neurotoxin is free of complexing proteins which naturally form complexes with botulinum neurotoxins. Moreover, the Examiner has failed to make the factual determinations set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966). Consequently, there is not adequate disclosure in the cited art to make out this exemplary rationale under the MPEP interpretation of *KSR*. Appellant submits that the Examiner has not met her burden to demonstrate that the rejected claims are *prima facie* obvious.

Impropriety of second grounds of rejection:

(B) Rejection of Claims 11-15 under 35 U.S.C. § 103(a) over *Carruthers, et al.* (Cosmetic Uses of Botulinum Toxin A Exotoxin, In: Klein AW, ed. *Tissue Augmentation in Clinical Practice: Procedures and Techniques.* New York: Marcel Dekker, 1998, p. 207-236) in view of *Heckman, et al.* (Arch. Dermatol., 1998, 134:1298-1299) and further in view of *Johnson, et al.* (U.S. Patent No. 5,512,547, published April 30, 1996).

The Examiner's rejection is based on the teaching of *Carruthers, et al.* of a method of treating cosmetic conditions, such as glabellar frown lines and horizontal forehead lines, (forms of wrinkles) by administering botulinum toxin A complex. The Examiner states on the record that *Carruthers, et al.* do not teach

the claim limitation, "the cosmetic wherein the cosmetic treatment is for hyperhidrosis (excessive sweating, a cosmetic condition)." (see the June 2, 2008 Office Action at page 10).

The Examiner cites *Heckman, et al.* for teaching injection of botulinum toxin for treating axillary hyperhidrosis. The Examiner states on the record that *Carruthers, et al.* and *Heckman, et al.* do not teach the claim limitation, "wherein the neurotoxins or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum neurotoxins and <u>wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes</u>." (see the November 5, 2007 Office Action at page 8 and the June 2, 2008 Office Action at page 9).

The Examiner cites *Johnson, et al.* for teaching a pharmaceutical composition comprising an essentially pure botulinum toxin A. The Examiner states on the record that, "*Johnson, et al.* teach that the use of the pure neurotoxin instead of the toxin complex, which is used commercially, reduced the amount of toxin required to obtain the necessary number of active units per vial as mandated by the Food and Drug Administration (column 2)", and that, "this improvement reduces the amount of inactive toxin in each vial and thereby lessens the possibility of antibody formation after injection of the preparation into patients (column 2)." (see the November 5, 2007 Office Action at page 8). Moreover, the Examiner states that *Johnson, et al.* teach that, "higher specific activity toxin preparations reduce the probability of patients developing neutralizing antibodies, and that it would be obviously desirable to have higher specific activity preparations (column 2)." (see the November 5, 2007 Office Action at page 8).

Appellant submits that the Examiner has not identified a prior art reference or combination of references which fulfill the basic requirements for establishing *prima facie* obviousness.

1. The Examiner has not established a proper case of *prima facie* obviousness; the Examiner has not established that all claim limitations are taught or suggested by the prior art.

1a. The claim limitation to a method of treating a human or animal with a cosmetic condition treatable with botulinum neurotoxin which is free of complexing proteins which naturally form complexes with botulinum neurotoxin complexes, "<u>wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxins</u>" is not taught or suggested by the prior art.

The Examiner states on the record that, "*Carruthers, et al.* and *Heckman, et al.* teach *do not* teach the claim limitation wherein the neurotoxin or mixture of neurotoxin is free of the complexing proteins which naturally form complexes with botulinum neurotoxins and <u>wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes.</u>" (Quote, see the Office Action of November 5, 2007 at page 8 and the Office Action of June 2, 2008 at page 9, *emphasis added*).

Appellant has already discussed that *Johnson, et al.* do not disclose or teach administration of a botulinum neurotoxin which is free from complexing proteins to humans or animals <u>already exhibiting neutralizing antibodies</u>. *Johnson, et al.* administer various *Clostridium botulinum* neurotoxin formulations to <u>naïve</u> animals to assess the <u>development</u> of antibodies and conclude that no neutralizing antibodies are formed in the subject animals when shelf-stable formulations of the invention are administered. (see *Johnson, et al.*, Columns 5-7 and Table 3).

Moreover, as discussed previously, *Johnson, et al.* teach that subjects who have developed neutralizing antibodies would <u>not</u> benefit from treatment with botulinum neurotoxins. *Johnson, et al.* state that, "The toxin is recognized by patient's immune systems as foreign and stimulates antibody production. This renders treatment of the various hyperactive muscle disorders with botulinum toxin <u>ineffective</u>." (see Column 1, lines 51-55). *Johnson, et al.* teach that administering *Clostridium botulinum* neurotoxin to patients who have developed neutralizing antibodies is futile, and thereby teaches away from administering botulinum neurotoxin to a subject who already exhibits neutralizing antibodies against botulinum neurotoxin complexes.

Appellant submits that *Carruthers, et al.*, *Heckman, et al.* and *Johnson, et al.* do not disclose or teach administration of botulinum neurotoxin to a human or animal, wherein the human or animal <u>already exhibits neutralizing antibodies against botulinum neurotoxin complexes</u>, a critical claim limitation for the reasons stated in the specification and noted above. Therefore, the Examiner's rejection should be reversed for failing to demonstrate all claim limitations to be taught or suggested by the art.

2. The Examiner has not established a proper rejection for *prima facie* obviousness by identifying a motivation to combine the teaching of the prior art references.

The obviousness rejection based on *Carruthers, et al.* in view of *Heckman, et al.* and further in view of *Johnson, et al.* is distinguished as per the previous discussion, in that the cited art do not teach administering botulinum neurotoxin which is free from complexing proteins which naturally form complexes with botulinum neurotoxins to a subject <u>already exhibiting neutralizing antibodies</u> against botulinum neurotoxin complexes.

The Examiner acknowledges that *Carruthers, et al.* and *Heckman, et al.* do not teach the instant claim limitation to administering botulinum neurotoxin wherein the neurotoxin or mixture of neurotoxin is free of the complexing proteins which naturally form complexes with botulinum neurotoxins and wherein the human or animal <u>already exhibits neutralizing antibodies against neurotoxin complexes</u>. (see the Office Action of November 5, 2007 at page 8). The Examiner attempts to cure this deficiency of disclosure by citing *Johnson, et al.* for teaching preparations of a purified botulinum neurotoxin which is free from complexing proteins. The Examiner concludes that it would be obvious to "substitute the botulinum toxin A (botulinum toxin A complex) in the method of treating patients with hyperhidrosis as taught by *Carruthers, et al.* and *Heckman, et al.* with the pure botulinum toxin A (without complexing proteins) as taught by *Johnson, et al.* because *Johnson, et al.* teach that purified product reduces the amount of inactive toxin in each vial and thereby lessens the possibility of antibody formation after injection of the preparation into patients." (see the Office Action of November 5, 2007 at pages 8-9).

Significantly and critically, *Carruthers, et al., Heckman, et al.* and *Johnson, et al.* do not teach or suggest the instant claim limitation to administration of a *Clostridium botulinum* neurotoxin which is free of complexing proteins <u>in subjects already exhibiting neutralizing antibodies</u>. *Johnson, et al.* teach the use of improved toxin preparations to lessen the possibility of antibody formation after injection into naïve subjects. This teaching, however, provides no correlation with the teaching that a botulinum neurotoxin preparation free from complexing proteins will be effective in treating subjects <u>already exhibiting neutralizing antibodies</u>, which is the subject of the instant invention.

In fact, the cited art teach that subjects who have developed neutralizing antibodies would <u>not</u> benefit from treatment with botulinum neurotoxin. *Johnson, et al.* state that, "The toxin is recognized by patient's immune systems as foreign

and stimulates antibody production. This renders treatment of the various hyperactive muscle disorders with botulinum toxin ineffective." (see Column 1, lines 51-55). The Examiner acknowledges that, "*Carruthers, et al.* teach that in neurologic patients, it is estimated that one-third of all treatment failures may be the result of the development of antibodies (page 214)." (see the November 5, 2007 Office Action at page 7). The cited art teach that administering a *Clostridium botulinum* neurotoxin to patients who have developed neutralizing antibodies is futile. Appellant submits that a method of treating subjects already exhibiting neutralizing antibodies would not have been obvious to one of ordinary skill in the art after consideration of all the facts.

Thus, the Examiner's position that, "It would be expected absent, evidence to the contrary, that a composition comprising pure botulinum toxin A (without complexing proteins) would be effective in treating patients that are nonresponders (have neutralizing antibodies to botulinum toxin A complex) because *Johnson, et al.* teach that higher specific activity preparations reduce the probability of patients developing neutralizing antibodies and it would be obviously desirable to have higher specific activity preparations than those currently available" finds no basis in the prior art disclosure of record.

Appellant submits that the Examiner has not fulfilled the obligations on the Office to establish a *prima facie* basis for alleging obviousness. It is well-established that the Office has the initial burden of establishing and factually and rationally supporting the conclusion that the claims are *prima facie* obvious; the Examiner's basis for obviousness cannot be sustained by mere conclusory statements.

Appellant submits that the factual information in the art of record regarding the ineffectiveness of botulinum neurotoxin treatment in subjects with neutralizing antibodies against botulinum neurotoxin complexes, as discussed above, does

not provide one skilled in the art with a reasonable expectation of success for treating a subject who already exhibits neutralizing antibodies against botulinum neurotoxin complexes with botulinum neurotoxin, wherein the neurotoxin is free of complexing proteins.

3. Examiner misapplied and fails to substantiate a finding of obviousness under *KSR International Co. v. Teleflex Inc.*

The Examiner recites exemplary rationales to support a finding of obviousness. The Examiner states that "Additionally, *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) discloses that if a technique has been used to improve one method and a person of ordinary skill would recognize that it would be used in similar methods in the same way, using the technique is obvious unless its application is beyond that person's skill." (see the June 2, 2008 Office Action at page 11). Furthermore, the Examiner states that, "*KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) also discloses that the combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." (see the June 2, 2008 Office Action at page 11).

Appellant rebuts the Examiner's conclusions that the instant invention is obvious based on the Examiner's limited reasoning. According to MPEP § 2143 interpreting *KSR*, the Examiner must demonstrate that the prior art disclose a "comparable" method which has been <u>improved in the same way as that method of the claimed invention</u>.

The Examiner states on the record that, "*Johnson, et* al. also recognized that there is a need in the art to solve the problem of the *development of neutralizing antibodies* to the botulinum toxin complex. *Johnson, et* al. provided a solution to this problem, by preparing a product that is a pure neurotoxin instead of the

complex." (Quote, page 11 of the June 2, 2008 Office Action, *emphasis added*). The improvement method of the cited art, according to the Examiner's reasoning is prevention of the development of neutralizing antibodies; which method has already been demonstrated on the record to be a method which is not comparable to the instant method of treating subjects who already exhibit neutralizing antibodies to botulinum neurotoxin complexes. (see Appellant's Response of January 3, 2008 at page 3).

The Examiner's basis for obviousness cannot be sustained by mere conclusory statements. The Examiner has not articulated reasoning within the KSR guidelines as to why it would be obvious to administer a botulinum neurotoxin to subjects already exhibiting neutralizing antibodies based on the teaching of preventing the development of neutralizing antibodies in *Johnson, et al.*

Furthermore, Appellant submits that the Examiner's second recitation of exemplary *KSR* bases for obviousness, namely that, "The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." (see the June 2, 2008 Office Action at page 11) is redundant on the last basis evaluated and no more substantiated. Absent demonstrated teaching that the improvement is a predictable use of prior art elements according to their established functions, Appellant rebuts the Examiner's rejection as being wholly without basis.

The third exemplary rationale recited by the Examiner as to why the claimed invention would have been obvious in view of *KSR* is that it would be, "obvious to try". (see the June 2, 2008 Office Action at page 12).

According to MPEP § 2143 interpretation of *KSR,* to reject the claims based on the "obvious to try" exemplary rationale to support a finding of obviousness,

the Examiner must articulate a finding that there had been a finite number of identified, <u>predictable</u> potential solutions to the recognized need or problem and that one of ordinary skill in the art could have pursued the known potential solutions with a <u>reasonable expectation of success</u>. Moreover, it is incumbent upon the Examiner to make the factual determinations set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966).

Appellant submits that the Examiner's third recitation of exemplary *KSR* bases for obviousness, namely that the instant method of treating subjects who already exhibit neutralizing antibodies against neurotoxin complexes with botulinum neurotoxin which is free of complexing proteins is "obvious to try", is not substantiated.

To begin, the Examiner has, in fact, identified NO predictable potential solutions to the problem of treating subjects who already exhibit neutralizing antibodies against neurotoxin complexes. As discussed above, the cited art teach that neutralizing antibodies directed against botulinum neurotoxin are the cause of therapeutic failure and that subjects who exhibit neutralizing antibodies would not benefit from treatment with botulinum neurotoxin, i.e., there are NO identified predictable potential solutions. Appellant has already discussed that *Carruthers, et al.* and *Johnson, et al.* teach that the presence of neutralizing antibodies directed against botulinum neurotoxin renders the toxin ineffective in subjects who exhibit neutralizing antibodies. The cited art teaching that the presence of neutralizing antibodies against botulinum neurotoxin complexes in a patient are the cause of therapeutic failure was also discussed in Appellant's previous responses. (see Appellant's Response of April 2, 2004 at page 4; Appellant's Response of July 27, 2007 at page 4; and Appellant's Response of January 3, 2008 at page 3) and the Examiner's admissions regarding *Carruthers, et al.*

The Examiner has, however, capriciously mischaracterized the cited disclosure, independently concluding that *Johnson, et al.* "solve the problem associated with patients that *have developed* neutralizing antibodies to botulinum toxin complex." (Quote, June 2, 2008 Office Action at page 13, *emphasis added*). The Examiner has not identified disclosure in *Johnson, et al.* in which *Johnson, et al.* solves, much less speaks to, problems associated with patients that have already developed neutralizing antibodies to botulinum toxin complex.

Appellant asserts that *Johnson, et al.*, as a whole, teaches that, "The toxin is recognized by patient's immune systems as foreign and stimulates antibody production. This renders treatment of the various hyperactive muscle disorders with botulinum toxin ineffective." (see Column 1, lines 51-55). Moreover, *Carruthers, et al.* teach that, "it is estimated that one third of all treatment failures may be the result of the development of neutralizing antibodies. (see page 214)." Appellant's method for treating subjects already exhibiting neutralizing antibodies would not have been obvious to one of ordinary skill in the art after consideration of the teaching of *Carruthers, et al.* and *Johnson, et al.*

Appellant submits that the teaching of the art of record does not provide a single predictable potential solution or any basis why one skilled in the art would have a reasonable expectation of success for treating a subject who already exhibits neutralizing antibodies against botulinum neurotoxin complexes with botulinum neurotoxin. Moreover, the Examiner has failed to make the factual determinations set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966). Consequently, there is not adequate disclosure in the cited art to make out this exemplary rationale under the MPEP interpretation of *KSR*. Appellant submits that the Examiner has not met her burden to demonstrate that the rejected claims are *prima facie* obvious.

Impropriety of the third grounds of rejection:

(C) **Rejection of Claims 16-18 under 35 U.S.C. § 103(a) over *Kessler, et al. (J. Neurol., 1999, 246:265-274) in view of Johnson, et al. (U.S. Patent No. 5,512,547, published April 30, 1996).***

The Examiner's rejection is based on the teaching of *Kessler, et al.* of a method of treating dystonias or a nervous system disorder treatable with a botulinum neurotoxin, such as cervical dystonia, with botulinum toxin type A complex. The Examiner states on the record that, *Kessler, et al.* do not teach the claim limitation "wherein the neurotoxins or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum neurotoxins and wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes." (see the November 5, 2007 Office Action at page 11).

The Examiner attempts to cure this deficiency of disclosure citing *Johnson, et al.* for teaching compositions of pure botulinum toxin which are free from non-complexing proteins. The Examiner concludes that, "*Johnson, et al.* teach the compositions of the invention reduces the amount of inactive toxin in each vial and thereby lessens the possibility of antibody formation after injection of the composition into patients. One of ordinary skill in the art would reasonably conclude that the compositions taught by *Johnson, et al.* comprise botulinum toxin formulations that lessen or reduce neutralizing antibodies because it contains essentially pure botulinum toxin." (see the June 2, 2008 Office Action at page 16).

Appellant submits that the Examiner has not identified a prior art reference or combination of references which fulfill the basic requirements for establishing *prima facie* obviousness.

1. The Examiner has not established a proper case of *prima facie* obviousness; the Examiner has not established that all claim limitations are taught or suggested by the prior art.

1a. The claim limitation to a method of treating a human or animal with a dystonia or a nervous system disorder treatable with a botulinum neurotoxin, wherein the neurotoxin is free of complexing proteins which naturally form complexes with botulinum neurotoxins, and "<u>wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes</u>" is not taught or suggested by the prior art.

The Examiner states on the record that *Kessler, et al.* <u>do not</u> teach the claim limitation, "wherein the neurotoxins or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum neurotoxins and <u>wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes</u>." (see the November 5, 2007 Office Action at page 11).

Appellant has already discussed that *Johnson, et al.* do not disclose or suggest administration of a botulinum neurotoxin which is free from complexing proteins to humans or animals who <u>already exhibit neutralizing antibodies</u>. *Johnson, et al.* administer various *Clostridium botulinum* neurotoxin formulations to <u>naïve</u> animals to assess the <u>development</u> of antibodies and conclude that no neutralizing antibodies are formed in the subject animals when shelf-stable formulations of the invention are administered (see Columns 5-7 and Table 3).

Significantly and critically, *Kessler, et al.* and *Johnson, et al.* do not teach or suggest the instant claim limitation to administration of *Clostridium botulinum* neurotoxin which is free of complexing proteins <u>in subjects already exhibiting</u>

neutralizing antibodies. In fact, the *Kessler, et al.* and *Johnson, et al.* disclosures teach that subjects who have developed neutralizing antibodies would not benefit from treatment with botulinum neurotoxin. *Johnson, et al.* state that, "The toxin is recognized by patient's immune systems as foreign and stimulates antibody production. This renders treatment of the various hyperactive muscle disorders with botulinum toxin ineffective." (see Column 1, lines 51-55). Moreover, *Kessler, et al.* teach that, "It is now well accepted that one of the major causes for the loss of treatment response is the formation of neutralizing serum antibodies to botulinum neurotoxin A. (see *Kessler, et al.* at page 272, right column).

Appellant's statements and evidence in the record of the instant application demonstrate the fact that both *Kessler, et al.* and *Johnson, et al.*, do not disclose or teach administering botulinum toxin compositions to a human or animal, "wherein the human or animal already exhibits neutralizing antibodies to botulinum toxin complexes", a critical claim limitation for the reasons stated in the specification and noted above.

2. The Examiner has not established a proper rejection for *prima facie* obviousness by identifying a motivation to combine the teaching of the prior art references.

The obviousness rejection based on *Kessler, et al.* in view of *Johnson, et al.* is distinguished as per the previous discussion, in that the cited art do not disclose or teach administering botulinum neurotoxin which is free from complexing proteins which naturally form complexes with botulinum neurotoxins to a subject already exhibiting neutralizing antibodies against botulinum neurotoxin complexes.

The Examiner's rejection is based on the teaching of *Kessler, et al.* of a method of treating dystonias or a nervous system disorder treatable with a

botulinum neurotoxin, such as cervical dystonia, with botulinum toxin type A complex. The Examiner states on the record that, *Kessler, et al.* do not teach the claim limitation "wherein the neurotoxins or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum neurotoxins and wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes." (see the November 5, 2007 Office Action at page 11). The Examiner cites *Johnson, et al.* for teaching compositions of pure botulinum toxin that are free from non-complexing proteins, concluding that it would be obvious to "substitute botulinum toxin A complex in the method of treating patients with cervical dystonia as taught by *Kessler, et al.* with the pure botulinum toxin A (without complexing proteins) as taught by *Johnson, et al.* because *Johnson, et al.* teach that purified product reduces the amount of inactive toxin in each vial and thereby <u>lessens the possibility of antibody formation</u> after injection of the preparation into patients." (see the November 5, 2007 Office Action at page 12).

Appellant does not dispute that *Johnson, et al.* developed compositions which are less antigenic and <u>lessen the possibility of antibody formation</u>, as acknowledged by the Examiner, which improvement overcomes the problem of <u>developing</u> neutralizing antibodies. To reject the claims for obviousness, the Examiner must establish that one skilled in the art actually has taught, or may infer from the combined disclosure of record, the administration of *Clostridium botulinum* neurotoxin which is free from complexing proteins for the efficacious treatment of subjects <u>already exhibiting neutralizing antibodies</u> against botulinum neurotoxin complexes.

Significantly and critically, *Kessler, et al.* and *Johnson, et al.* do not teach or suggest the instant claim limitation to administration of *Clostridium botulinum* neurotoxin which is free of complexing proteins <u>in subjects already exhibiting neutralizing antibodies</u>.

In fact, the cited art teach that subjects who have developed neutralizing antibodies would not benefit from treatment with botulinum neurotoxin. *Johnson, et al.* state that, "The toxin is recognized by patient's immune systems as foreign and stimulates antibody production. This renders treatment of the various hyperactive muscle disorders with botulinum toxin ineffective." (see Column 1, lines 51-55). The Examiner acknowledges that, "*Kessler, et al.* teach that secondary nonresponse is one of the major problems in long-term treatment of CD with botulinum toxin A because it entails discontinuing, depriving the patent of the most successful therapy available (page 272)." (see the November 5, 2007 Office Action at page 11). *Kessler, et al.* states that, "It is now well accepted that one of the major causes for the loss of treatment response is the formation of neutralizing serum antibodies to botulinum neurotoxin A. (see page 272, right column). The cited art teach that administering a *Clostridium botulinum* neurotoxin to patients who have developed neutralizing antibodies is futile. Appellant submits that the instant method of treating subjects already exhibiting neutralizing antibodies would not have been obvious to one of ordinary skill in the art after consideration of all the facts.

Thus, the Examiner's conclusion that, "*Johnson, et al.* recognize patients that have developed neutralizing antibodies to the complex are a growing concern in the art, thus this is the very bases for the development of compositions comprising essentially pure botulinum toxin. *Therefore, it would be obvious to administer these compositions to patients that have developed neutralizing antibodies to the botulinum toxin complex*" (see the Office Action of June 2, 2008 at page 16, *emphasis added*), finds no basis in the prior art disclosure of record.

Appellant submits that the Examiner has not provided any basis in fact or technical reasoning as to why it would be obvious to administer a botulinum neurotoxin to a subject already exhibiting neutralizing antibodies based on the

teaching of preventing the development of neutralizing antibodies, as taught in *Johnson, et al.* The *Johnson, et al.* teaching of preventing the development of neutralizing antibodies provides no correlation with the teaching that a botulinum neurotoxin preparation free from complexing proteins will be effective in treating subjects already exhibiting neutralizing antibodies, which is the subject of the instant invention.

Appellant submits that the factual information in the art of record regarding unsuccessful botulinum neurotoxin treatment in subjects exhibiting neutralizing antibodies against botulinum neurotoxin complexes, as discussed above, rebuts the Examiner's conclusion that, "*Therefore, it would be obvious to administer these compositions to patients that have developed neutralizing antibodies to the botulinum toxin complex*". The Examiner's conclusion finds no legitimate basis in the citations of record and amounts to nothing more than opinion and speculation of the Examiner.

3. Examiner misapplied and fails to substantiate a finding of obviousness under *KSR International Co. v. Teleflex Inc.*

The Examiner recites exemplary rationales to support a finding of obviousness. The Examiner states that "Additionally, *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) discloses that if a technique has been used to improve one method and a person of ordinary skill would recognize that it would be used in similar methods in the same way, using the technique is obvious unless its application is beyond that person's skill." (see page 16 of the June 2, 2008 Office Action). Furthermore, the Examiner states that, "*KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) also discloses that the combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." (see the June 2, 2008 Office Action at page 16).

Appellant rebuts the Examiner's conclusions that the instant invention is obvious based on the Examiner's limited reasoning. According to MPEP § 2143 interpreting *KSR*, the Examiner must demonstrate that the prior art contain a "comparable" method which has been improved in the same way as that method of the claimed invention.

The Examiner states on the record that, "*Johnson, et al*. also recognized that there is a need in the art to solve the problem of the *development of neutralizing antibodies* to the botulinum toxin complex. *Johnson, et al*. provided a solution to this problem, by preparing a product that is a pure neurotoxin instead of the complex." (Quote, page 17 of the June 2, 2008 Office Action, *emphasis added*). The improvement method of the cited art, according to the Examiner's limited reasoning is prevention of the development of neutralizing antibodies; which method has already been demonstrated on the record to be a method which is not comparable to the instant method of treating subjects who already exhibit neutralizing antibodies to botulinum neurotoxin complexes. (see Appellant's Response of January 3, 2008 at page 3).

Moreover, the Examiner concludes that, "Thus, it would be obvious to administer an essential pure composition of botulinum toxin (e.g. free of complexing proteins) to patients that have neutralizing antibodies because the composition of essentially pure botulinum toxin was developed to lessen or reduce the amount of neutralizing antibodies produced in patients after administration of the composition." (see the June 2, 2008 Office Action at page 17). The Examiner has not provided any basis in fact or technical reasoning as to why it would be obvious to administer a botulinum neurotoxin which is free from complexing proteins to a subject who already exhibits neutralizing antibodies based on the teaching of preventing the development of neutralizing antibodies in *Johnson, et al.*

Appellant submits that the Examiner's second recitation of exemplary *KSR* bases for obviousness, namely that, "The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." is redundant on the last basis evaluated and no more substantiated. Absent demonstrated teaching that the improvement is more than a predictable use of prior art elements according to their established functions, Appellant rebuts the Examiner's rejection as without basis.

The third exemplary rationale recited by the Examiner as to why the claimed invention would have been obvious in view of *KSR* is that it would be, "obvious to try". (see the June 2, 2008 Office Action at page 17).

According to MPEP § 2143 interpretation of *KSR,* to reject the claims based on the "obvious to try" exemplary rationale to support a finding of obviousness, the Examiner must articulate a finding that here had been a finite number of identified, <u>predictable</u> potential solutions to the recognized need or problem and that one of ordinary skill in the art could have pursued the known potential solutions with a <u>reasonable expectation of success</u>. Moreover, it is incumbent upon the Examiner to make the factual determinations set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966).

Appellant submits that the Examiner's third recitation of exemplary *KSR* bases for obviousness, namely that the instant method of treating subjects who already exhibit neutralizing antibodies against neurotoxin complexes with botulinum neurotoxin which is free of complexing proteins is "obvious to try", is not substantiated.

To begin, the Examiner has, in fact, identified NO predictable potential solutions to the problem of treating subjects who already exhibit neutralizing

antibodies against neurotoxin complexes. As discussed above, the cited art teach that neutralizing antibodies directed against botulinum neurotoxin are the cause of therapeutic failure and that subjects who exhibit neutralizing antibodies would not benefit from treatment with botulinum neurotoxin, i.e., there are NO identified predictable potential solutions. Appellant has already discussed that *Kessler, et al.* and *Johnson, et al.* teach that the presence of neutralizing antibodies directed against botulinum neurotoxin renders the toxin ineffective in subjects who exhibit neutralizing antibodies. The art of record teaching that neutralizing antibodies against botulinum neurotoxin complexes are the cause of therapeutic failure was also discussed in Appellant's previous responses. (see Appellant's Response of April 2, 2004 at page 4; Appellant's Response of July 27, 2007 at page 4; and Appellant's Response of January 3, 2008 at page 3).

The Examiner has, however, capriciously mischaracterized the cited disclosure, independently concluding that, *Johnson, et al.* "solve the problem associated with patients that *have developed* neutralizing antibodies to botulinum toxin complex" (Quote, June 2, 2008 Office Action at page 18, *emphasis added*). The Examiner has not identified disclosure in *Johnson, et al.* in which *Johnson, et al.* solves, much less speaks to, problems associated with patients who have already developed neutralizing antibodies to botulinum toxin complex.

Appellant asserts that *Johnson, et al.*, as a whole, teaches that, "The toxin is recognized by patient's immune systems as foreign and stimulates antibody production. This renders treatment of the various hyperactive muscle disorders with botulinum toxin ineffective." (see Column 1, lines 51-55). Moreover, *Kessler, et al.* teach that, "It is now well accepted that one of the major causes for the loss of treatment response is the formation of neutralizing serum antibodies to botulinum neurotoxin A. Appellant's method for treating subjects already exhibiting neutralizing antibodies would not have been obvious to one of ordinary

skill in the art after consideration of the teaching of *Kessler, et al.* and *Johnson, et al.*

Appellant submits that the teaching of the art of record does not provide a single predictable potential solution or any basis why one skilled in the art would have a reasonable expectation of success for treating a subject who already exhibits neutralizing antibodies against botulinum neurotoxin complexes with botulinum neurotoxin. Moreover, the Examiner has failed to make the factual determinations set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966). Appellant submits that the Examiner has not met her burden to demonstrate that the rejected claims are *prima facie* obvious. Consequently, there is not adequate disclosure in the cited art to make out this exemplary rationale under the MPEP interpretation of *KSR*.

(D) **Rejection of Claims 16-18 under 35 U.S.C. § 103(a) over *Göschel, et al.* (*Experimental Neurology 1997, 147:96-102*) in view of *Johnson, et al.* (*U.S. Patent No. 5,512,547, published April 30, 1996*).**

The Examiner's rejection is based on the teaching of *Göschel, et al.* of administering botulinum toxin in a method of treating a dystonia, or a nervous system disorder treatable with botulinum toxin. The Examiner states on the record that *Göschel, et al.* do not teach the claim limitation, "wherein the neurotoxins or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum neurotoxins and wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes." (see the November 5, 2007 Office Action at page 14 and the June 2, 2008 Office Action at page 19).

The Examiner cites *Johnson, et al.* for teaching compositions of pure botulinum toxin that are free from non-complexing proteins. The Examiner states

on the record that, "*Johnson, et al.* teach the composition of the invention reduces the amount of inactive toxin in each vial and thereby lessens the possibility of antibody formation after injection of the composition into patients." (see the June 2, 2008 Office Action at page 21).

Appellant submits that the Examiner has not identified a prior art reference or combination of references which fulfill the basic requirements for establishing *prima facie* obviousness.

1. The Examiner has not established a proper case of *prima facie* obviousness; the Examiner has not established that all claim limitations are taught or suggested by the prior art.

1a. The claim limitation to a method of treating a human or animal with a dystonia or a nervous system disorder treatable with a botulinum neurotoxin, wherein the neurotoxin is free of complexing proteins which naturally form complexes with botulinum neurotoxins, and "<u>wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes</u>" is not taught or suggested by the prior art.

Appellant has already demonstrated that *Johnson, et al.* do not disclose or suggest administration of a botulinum neurotoxin which is free from complexing proteins to humans or animals who <u>already exhibit neutralizing antibodies</u>.

The Examiner states on the record that *Göschel, et al.* do not teach the claim limitation, "wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes" (see the November 5, 2007 Office Action at page 14 and the June 2, 2008 Office Action at page 19).

Therefore, the Examiner's rejection should be reversed for failing to demonstrate all claim limitations to be taught or suggested by the art. Absent such showing, the Examiner has not met her burden to demonstrate that the rejected claims are *prima facie* obvious.

2. The Examiner has not established a proper rejection for *prima facie* obviousness by identifying a motivation to combine the teaching of the prior art references.

The obviousness rejection based on *Göschel, et al.* in view of *Johnson, et al.* is distinguished as per the previous discussion, in that the cited art do not teach administering botulinum neurotoxin, which is free from complexing proteins which naturally form complexes with botulinum neurotoxins, to a subject who already exhibits neutralizing antibodies against botulinum neurotoxin complexes.

The Examiner acknowledges that *Göschel, et al.* do not teach the claim limitation, "wherein the neurotoxins or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum neurotoxins and wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes." (see the November 5, 2007 Office Action at page 14 and the June 2, 2008 Office Action at page 19).

The Examiner attempts to cure this deficiency of disclosure by citing *Johnson, et al.* for teaching preparations of a purified botulinum neurotoxin which is free from complexing proteins. The Examiner concludes that, "*Johnson, et al.* recognizes patient that have developed neutralizing antibodies to the complex are a growing concerning the art, thus this is the very bases for the development of compositions comprising "essentially pure botulinum toxin". *Therefore, it would be obvious to administer these compositions to patients that have*

developed neutralizing antibodies to the botulinum toxin complex." (see the June 2, 2008 Office Action at page 22, *emphasis added*).

Significantly and critically, *Göschel, et al.* and *Johnson, et al.* do not teach or suggest the instant claim limitation to administration of *Clostridium botulinum* neurotoxin which is free of complexing proteins <u>in subjects already exhibiting neutralizing antibodies</u>. *Johnson, et al.* teach the use of improved toxin preparations to lessen the possibility of antibody formation after injection into naïve subjects. This teaching, however, provides no correlation with the teaching that a botulinum neurotoxin preparation free from complexing proteins will be effective in treating subjects <u>already exhibiting neutralizing antibodies</u>, which is the subject of the instant invention.

In fact, the cited art teach that subjects who have developed neutralizing antibodies would not benefit from treatment with botulinum neurotoxin. *Johnson, et al.* state that, "The toxin is recognized by patient's immune systems as foreign and stimulates antibody production. This renders treatment of the various hyperactive muscle disorders with botulinum toxin <u>ineffective</u>." (see Column 1, lines 51-55). The Examiner acknowledges that *Göschel, et al.* teach that, "neutralizing antibodies were found in the sera of all non-responders (patients that have developed neutralizing antibodies against botulinum toxin A) (pages 98-99)" and that, "neutralizing antibodies were the cause of therapeutic failure (page 101)." (see the Office Action of January 13, 2004 at page 8; the Office Action of July 9, 2004 at page 4; the Office Action of November 5, 2007 at page14; and the Office Action of June 2, 2008 at page 19). The cited art teach that administering a *Clostridium botulinum* neurotoxin to patients who have developed neutralizing antibodies is futile. Appellant submits that a method of treating subjects already exhibiting neutralizing antibodies with botulinum neurotoxin would not have been obvious to one of ordinary skill in the art after consideration of all the facts.

Thus, the Examiner's position that, "*Johnson, et al.* recognize patients that have developed neutralizing antibodies to the complex are a growing concern in the art, thus this is the very bases for the development of compositions comprising essentially pure botulinum toxin. *Therefore, it would be obvious to administer these compositions to patients that have developed neutralizing antibodies to the botulinum toxin complex*" (see the Office Action of June 2, 2008 at page 22, *emphasis added*), finds no basis in the prior art disclosure of record.

Appellant submits that the Examiner has not fulfilled the obligations on the Office to establish a *prima facie* basis for alleging obviousness. It is well-established that the Office has the initial burden of establishing and <u>factually and rationally</u> supporting the conclusion that the claims are *prima facie* obvious; the Examiner's basis for obviousness cannot be sustained by mere conclusory statements.

Appellant submits that the <u>factual information</u> in the art of record regarding the ineffectiveness of botulinum neurotoxin treatment in subjects with neutralizing antibodies against botulinum neurotoxin complexes, as disclosed above, demonstrates that the Examiner's position that, it would be obvious to administer compositions of essentially pure botulinum toxin to patients that have developed neutralizing antibodies to the botulinum toxin complex, is without basis.

3. Examiner misapplied and fails to substantiate a finding of obviousness under *KSR International Co. v. Teleflex Inc.*

The Examiner recites exemplary rationales to support a finding of obviousness. The Examiner states that "Additionally, *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) discloses that if a technique has been used to improve one method and a person of ordinary skill would recognize that it

would be used in similar methods in the same way, using the technique is obvious unless its application is beyond that person's skill." (see the June 2, 2008 Office Action at page 22). Furthermore, the Examiner states that, "*KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) also discloses that the combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." (see the June 2, 2008 Office Action at page 22).

Appellant rebuts the Examiner's conclusions that the instant invention is obvious based on the Examiner's limited reasoning. According to MPEP § 2143 interpreting *KSR*, the Examiner must demonstrate that the prior art contain a "comparable" method which has been <u>improved in the same way as that method of the claimed invention</u>.

The Examiner states on the record that, "*Johnson, et a*l. also recognized that there is a need in the art to solve the problem of the *development of neutralizing antibodies* to the botulinum toxin complex. *Johnson, et al.* provided a solution to this problem, by preparing a product that is a pure neurotoxin instead of the complex." (Quote, page 22 of the June 2, 2008 Office Action, *emphasis added*). The improvement method of the cited art, according to the Examiner's limited reasoning is <u>prevention of the development of neutralizing antibodies</u>; which method has already been demonstrated on the record to be a method which is not comparable to the instant method of treating subjects who <u>already exhibit neutralizing antibodies</u> to botulinum neurotoxin complexes. (see Appellant's Response of April 2, 2004 pages 3-4; Appellant's Response of July 27, 2005 at pages 3-4; and Appellant's Response of January 3, 2008 at page 3).

The Examiner concludes that, "Thus, it would be obvious to administer an essential pure composition of botulinum toxin (e.g. free of complexing proteins) to patients that have neutralizing antibodies because the composition of essentially

pure botulinum toxin was developed to lessen or reduce the amount of neutralizing antibodies produced in patients after administration of the composition." (see the June 2, 2008 Office Action at pages 22-23).

Appellant submits that the Examiner's conclusion is precisely the product of an impermissible hindsight reconstruction. The Examiner has not provided any basis in fact or technical reasoning as to why it would be obvious to administer a botulinum neurotoxin to a subject who <u>already exhibits neutralizing antibodies</u> based on the teaching of <u>preventing the development</u> of neutralizing antibodies, as taught in *Johnson, et al.*

Appellant submits that the Examiner's second recitation of exemplary *KSR* bases for obviousness, namely that, "The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results." is redundant on the last basis evaluated and no more substantiated. Appellant rebuts the Examiner's rejection as without basis.

The third exemplary rationale recited by the Examiner as to why the claimed invention would have been obvious in view of *KSR* is that it would be, "obvious to try". (see the June 2, 2008 Office Action at page 23).

According to MPEP § 2143 interpretation of *KSR,* to reject the claims based on the "obvious to try" exemplary rationale to support a finding of obviousness, the Examiner must articulate a finding that here had been a finite number of identified, <u>predictable</u> potential solutions to the recognized need or problem and that one of ordinary skill in the art could have pursued the known potential solutions with a <u>reasonable expectation of success</u>. Moreover, the Examiner is instructed to make the factual determinations set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966).

Appellant submits that the Examiner's third recitation of exemplary *KSR* bases for obviousness, namely that the instant method of treating subjects who already exhibit neutralizing antibodies against neurotoxin complexes with botulinum neurotoxin which is free of complexing proteins is "obvious to try", is not substantiated.

To begin, the Examiner has, in fact, identified NO predictable potential solutions to the problem of treating subjects who already exhibit neutralizing antibodies against neurotoxin complexes. As discussed above, the cited art teach that neutralizing antibodies directed against botulinum neurotoxin are the cause of therapeutic failure and that subjects who exhibit neutralizing antibodies would not benefit from treatment with botulinum neurotoxin, i.e., there are NO identified predictable potential solutions.

The mere fact that references can be combined or modified does not render the resulting combination obvious unless the results would have been predictable to one skilled in the art. *KSR International Co. v. Teleflex Inc., 127 S. Ct. 1727, 1740-41, 82 USPQ2d 1385, 1396 (2007).* The Court recognized that it is often necessary to look at the interrelated teachings of multiple references in order to determine whether there is an apparent reason to combine the known elements in the fashion claimed. *Id. at 1740-41*

Appellant submits that one skilled in the art could not reasonably predict from the teaching of <u>reducing the probability of antibody formation</u> in a patient, as taught in *Johnson, et al.,* that a botulinum neurotoxin which is free from complexing proteins which naturally form complexes with botulinum neurotoxins, would be effective in patients who <u>already exhibit neutralizing antibodies against botulinum neurotoxin complexes</u>.

The Examiner has identified prior art teaching that administering botulinum neurotoxin in a subject who has neutralizing antibodies is ineffective. The Examiner acknowledges that, "*Göschel, et al.* teach that some patients develop neutralizing antibodies and these neutralizing antibodies cause therapeutic failure (these patients are non-responders) (see the June 2, 2008 Office Action at page 21), and that *Göschel, et al.* teach that, "neutralizing antibodies were found in the sera of all non-responders (patients that have developed neutralizing antibodies against botulinum toxin A) (pages 98-99)" and that, "neutralizing antibodies were the cause of therapeutic failure (page 101)." (see the Office Action of January 13, 2004 at page 8; the Office Action of July 9, 2004 at page 4; the Office Action of November 5, 2007 at page14; and the Office Action of June 2, 2008 at page 19).

Appellant has already demonstrated that both *Göschel, et al.* and *Johnson, et al.* teach that the presence of neutralizing antibodies directed against botulinum neurotoxin renders the toxin <u>ineffective</u> in subjects who exhibit neutralizing antibodies. The cited art teaching that neutralizing antibodies against neurotoxin complexes is the cause of therapeutic failure was also discussed in Appellant's previous responses. (see Appellant's Response of April 2, 2004 at page 4; Appellant's Response of July 27, 2007 at page 4; and Appellant's Response of January 3, 2008 at page 3).

The Examiner has, however, capriciously mischaracterized the cited disclosure, independently concluding that *Johnson, et al.* "solve the problem associated with patients that <u>*have developed*</u> neutralizing antibodies to botulinum toxin complex." (Quote, June 2, 2008 Office Action at page 24, *emphasis added*). The Examiner has not identified disclosure in *Johnson, et al.* in which *Johnson, et al.* solves, much less speaks to, problems associated with patients that <u>have already developed</u> neutralizing antibodies to botulinum toxin complex.

Appellant asserts that *Johnson, et al.*, as a whole, teaches that, "The toxin is recognized by patient's immune systems as foreign and stimulates antibody production. <u>This renders treatment of the various hyperactive muscle disorders with botulinum toxin ineffective</u>." (see Column 1, lines 51-55). Appellant's claimed method of treating subjects already exhibiting neutralizing antibodies would not have been obvious to one of ordinary skill in the art after consideration of all the facts.

Appellant submits that the Examiner has not fulfilled the obligations on the Office to establish a *prima facie* basis for alleging obviousness.

4. Conclusion

In summary, Appellant submits that the Examiner's continued rejection of the claims for obviousness, despite Appellant's repeated demonstrations that the cited art do not teach all claim limitations and that the cited art do not motivate one skilled in the art to combine the references' teaching, is based upon a faulty understanding of the well-established case law and the statutory function of the claims as well as an arbitrary and capricious misinterpretation of the art of record. From this faulty legal analysis, the Examiner fails to give proper and due consideration to Appellant's rebuttal arguments and evidence which, when properly considered as required by the case law, rebut any *prima facie* rejection for obviousness.

Therefore, Appellant submits that the Examiner's obviousness rejections are improper and should be reversed. Allowance is solicited.

If necessary, the Commissioner is hereby authorized in this, to charge any further or additional fees which may be required (due to omission, deficiency, or otherwise), or to credit any overpayment, to Deposit Account No. 08,3220.

Respectfully submitted:

THE FIRM OF HUESCHEN AND SAGE

By: G. Patrick Sage

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VIII. CLAIMS APPENDIX

Listing of Claims Involved in the Appeal.

Claims 1-10: (canceled)

Claim 11. (previously presented) A method of treating a human or animal with a cosmetic condition treatable with a botulinum neurotoxin, comprising administration, to the human or animal, a treatment effective amount of a botulinum neurotoxin from *Clostridium botulinum* of type A, B, C, D, E, F or G or a mixture of two or more botulinum neurotoxins, wherein the neurotoxin or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum neurotoxins, and wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes.

Claim 12. (previously presented) The method of claim 11 wherein the subject exhibits neutralizing antibodies against a complex of *Clostridium botulinum* type A or B or a complex of *Clostridium botulinum* type A and type B.

Claim 13. (previously presented) The method of claim 11 wherein the cosmetic treatment is for hyperhidrosis.

Claim 14. (previously presented) The method of claim 11 wherein the cosmetic treatment is for wrinkling.

Claim 15. (previously presented) The method of claim 14 wherein the cosmetic treatment is for facial wrinkling.

Claim 16. (previously presented) A method of treating a human or animal with dystonia or a nervous system disorder treatable with a botulinum neurotoxin, comprising administration, to the human or animal, a treatment effective amount of a botulinum neurotoxin from *Clostridium botulinum* of type A, B, C, D, E, F or G or a mixture of two or more botulinum neurotoxins, wherein the neurotoxin or mixture of neurotoxins is free of the complexing proteins which naturally form complexes with botulinum neurotoxins, and wherein the human or animal already exhibits neutralizing antibodies against botulinum neurotoxin complexes.

Claim 17. (previously presented) The method of claim 16 wherein the subject exhibits neutralizing antibodies against a complex of *Clostridium botulinum* type A or B or a complex of *Clostridium botulinum* type A and type B.

Claim 18. (previously presented) The method of claim 16 wherein the dystonia or disorder of the nervous system is selected from spasmodic torticollis, blepharospasm, spasticities such as footdrop, hemifacial spasms, migraine, low back pain, cervical spine disorders and hypersalivation.

IX. EVIDENCE APPENDIX

A. Copy of *Johnson, et al.*, U.S. Patent No. 5,512,547, published April 30, 1996).

Johnson, et al. was entered in the record by the examiner in the Office Action dated November 5, 2007.

B. Copy of *Keen, et al.* (Plastic and Reconstructive Surgery, July 1994, 94:94-99).

Keen, et al. was entered in the record by the examiner in the Office Action dated January 13, 2004.

C. Copy of *Carruthers, et al.* (Cosmetic Uses of Botulinum Toxin A Exotoxin, In: Klein AW, ed. *Tissue Augmentation in Clinical Practice: Procedures and Techniques*. New York: Marcel Dekker, 1998, p. 207-236).

Carruthers, et al. was entered in the record by the examiner in the Office Action dated August 1, 2006.

D. Copy of *Heckman, et al.* (Arch. Dermatol., 1998, 134:1298-1299).

Heckman, et al. was entered in the record by the examiner in the Office Action dated November 5, 2007.

E. Copy of *Kessler, et al.* (J. Neurol., 1999, 246:265-274).

Kessler, et al. was entered in the record by the examiner in the Office Action dated November 5, 2007.

F. Copy of *Göschel, et al.* (Experimental Neurology 1997, 147:96-102).

Göschel, et al. was entered in the record by the examiner in the Office Action dated January 13, 2004.

X. RELATED PROCEEDINGS APPENDIX

Not Applicable – None.

Applicant: Hans BIGALKE and Jürgen FREVERT
Application Serial No. 10/018,373
Title: THERAPEUTIC COMPOSITION COMPRISING A BOTULINUM NEUROTOXIN
Filed: December 6, 2001

CERTIFICATE OF MAILING BY EXPRESS MAIL SERVICE UNDER 37 CFR § 1.10

Express Mail No.:EM 020042203 US

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Appeal Brief under 37 CFR § 41.37, fifty-five (55) pages, and accompanying references; Fee Transmittal Form and Fee under 37 CFR § 41.20(b)(2) in the form of a check in the amount of $540.00; and Return Receipt Postcard is being deposited on October 7, 2008 with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR § 1.10 as Express Mail No. EM 020042203 US in an envelope addressed to:

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US005512547A

United States Patent [19]

Johnson et al.

[11] **Patent Number: 5,512,547**

[45] **Date of Patent: Apr. 30, 1996**

[54] **PHARMACEUTICAL COMPOSITION OF BOTULINUM NEUROTOXIN AND METHOD OF PREPARATION**

[75] Inventors: **Eric A. Johnson; Michael C. Goodnough**, both of Madison, Wis.

[73] Assignee: **Wisconsin Alumni Research Foundation**, Madison, Wis.

[21] Appl. No.: **322,624**

[22] Filed: **Oct. 13, 1994**

[51] **Int. Cl.**[6] **A61K 38/00**; C07K 1/00

[52] **U.S. Cl.** **514/21**; 514/2; 530/350; 530/363; 530/364; 530/825

[58] **Field of Search** 514/21.2; 530/350, 530/363, 364, 825

[56] **References Cited**

PUBLICATIONS

Goodnough et al, *Applied and Environmental Microbiology*, vol. 58, No. 10, pp. 3426–3428, Oct. 1992.

Schantz, E. J. and Scott, A. B., Biomedical Aspects of Botulism, Academic Press, Inc., pp.143–150 (1981).

Schantz, E. J., and Kautter, D. A., *Journal of the Association of Official Analytical Chemists*, vol. 61, pp. 96–99 (1978).

Melling et al., "Clostridium Botulinum Toxins: Nature and Preparation for Clinical Use." Eye, vol. 2, 1988, pp.–16–23.

Hambleton et al. "Production, Purification and Toxoiding of Clostridium Botulinum Type A Toxin", Biomedical Aspects of Botulism, Academic Press, Inc., (1981) pp. 247–260.

Primary Examiner—Christina Y. Chan
Assistant Examiner—Abdel A. Mohamed
Attorney, Agent, or Firm—Quarles & Brady

[57] **ABSTRACT**

Pharmaceutical compositions of botulinum neurotoxin containing higher specific toxicity and increased stability at higher temperatures than currently available preparations.

3 Claims, No Drawings

PHARMACEUTICAL COMPOSITION OF BOTULINUM NEUROTOXIN AND METHOD OF PREPARATION

FIELD OF THE INVENTION

The present invention relates to botulinum toxin. More particularly, it relates to novel pharmaceutical compositions containing botulinum toxin and a method for preparing the compositions.

BACKGROUND OF THE INVENTION

The most serious form of bacterial food poisoning is botulism which is caused by neurotoxins produced by *Clostridium botulinum*. The toxins are usually preformed by the causative organism in foods and subsequently absorbed through the intestinal tract and transported via the circulatory system to motor nerve synapses where their action blocks normal neural transmissions. Various serotypes of *C. botulinum* produce neurotoxins with similar toxic activity but which differ antigenically. Serotype A toxin is the predominant cause of botulism in the United States while type B toxin is the most prevalent in Europe.

Crystalline type A botulinum toxin complex was prepared in 1979 by E. J. Schantz of the Food Research Institute/Department of Food Microbiology and Toxicology at the University of Wisconsin-Madison. It has been used medically to treat hyperactive muscle disorders such as strabismus, blepharospasm, and spasmodic torticollis. Treatment involves injection of nanogram quantities of the toxin directly into the hyperactive muscles. The toxin inhibits the release of acetylcholine across the synaptic junction causing a decrease in the activity of the injected muscles.

Type A neurotoxin produced by *C. botulinum* is present as part of a complex of at least seven different noncovalently bound proteins. High quality type A toxin complex has a specific toxicity of 3×10^7 mouse intraperitoneal 50% lethal doses (LD_{50}) per mg. The purified neurotoxin, that is the neurotoxin that has been chromatographically separated from the other proteins of the toxin complex, has a specific toxicity of 9×10^7 to 1×10^8 LD_{50} per mg. In the medical field, a unit (U) is considered to be 1 LD_{50}. Toxin titers are determined in female, white mice, 18–22g in weight according to the method of Schantz and Kautter as described in Association of Official and Analytical Chemistry, vol. 61, p. 96, (1978).

A major drawback to the use of botulinum toxin in treatment of hyperactive muscle disorders is development of antibodies or other types of immunity by patients. The toxin is recognized by patient's immune systems as foreign and stimulates antibody production. This renders treatment of the various hyperactive muscle disorders with botulinum toxin ineffective. One way to reduce the number of patients developing neutralizing antibodies would be to develop a more shelf-stable product with a higher specific activity following lyophilization. Such a formulation would result in a product that is not as antigenic as the currently available product and lesser quantities of toxin would be required for treatment.

Botulinal toxin is very susceptible to denaturation due to surface denaturation, heat, and alkaline conditions. Lyophilization or freeze-drying of botulinal toxin is the most economically sound and practical method of distributing the product in a form that is stable and readily used by the clinician. The current commercial type A botulinal toxin product is made by combining up to 500 ng/ml of type A toxin complex in 5.0 mg/ml human serum albumin (HSA) with 9.0 mg/ml sodium chloride at a pH of 7.3. After dissolution, 0.1 ml is dried to obtain 100±30 active U of toxin, 0.5 mg of HSA, and 0.9 mg of sodium chloride per vial. This product has a saline concentration of 0.9% when reconstituted in 1.0 ml of dH_2O. The current commercial formulation which employs the toxin complex has a specific toxicity of about 2.5 U/ng after drying BOTOX®, crystalline botulinum toxin complex, Allergon, Inc. of Irvine, Calif. The considerable loss (up to 90%) of activity during drying causes the formation of inactive toxin that serves as a toxoid inciting antibody formation.

A rabbit model in which repetitive injections of various type A toxin preparations have been given to simulate the treatment of a focal dystonia has been used to assess the immunogenicity of various toxin preparations. The model consists of injecting albino rabbits with sublethal doses of the toxin over a period of time and assaying the serum of the animals for the ability to neutralize a small but carefully quantitated amount of purified type A toxin. Our results show that the product presently available in the United States which has the lowest specific toxicity of all preparations tested is the most antigenic of all the preparations tested to date. These results indicate that high specific activity preparations reduce the probability of patients developing neutralizing antibodies. It obviously would be desirable to have higher specific activity preparations than those currently available.

The current commercial product must be stored at a temperature of −10° C. or less to maintain the labelled potency for the one year shelf life. It also would be advantageous to have a product that could be stored at higher temperatures (i.e. room temperature). This would facilitate more practical shipping and storage of the toxin.

BRIEF SUMMARY OF THE INVENTION

We have discovered that pharmaceutical compositions made from a liquid formulation containing essentially pure botulinum type A neurotoxin, human serum albumin (HSA), and trehalose provides for the improved recovery of active toxin following lyophilization (>80%). The use of the pure neurotoxin instead of the toxin complex, which is used commercially, reduces the amount of toxin required to obtain the necessary number of active U per vial as mandated by the U.S. Food and Drug Administration. This improvement also reduces the amount of inactive toxin (toxoid) in each vial and thereby lessens the possibility of antibody formation after injection of the preparation into patients.

We also have discovered that the compositions obtained by adding trehalose to the pre-lyophilization formula increases the glass transition temperature of the dried material and thereby increases the usable storage temperature. The addition of the trehalose surprisingly enhances the temperature stability of the dried toxin and lessens both the risk of loss in potency with corresponding degradation during storage and shipment with a consequent increase in antigenic potential due to temperature abuse. In addition to trehalose, we have discovered that the polyhydroxy compound maltotriose and possibly other polyols can be used.

DESCRIPTION OF PREFERRED EMBODIMENT

The preferred pharmaceutical compositions of the present invention have the following composition:

Botulinum Type A Neurotoxin (>95% purity)
Trehalose, 10 mg/vial
Serum albumin, 0.5 mg/vial
Water for Injection, 0.1–0.5 ml

In addition to human serum albumin, other known stabilizing proteins including bovine serum albumin, can be used in the compositions of the present invention.

The Hall A strain of type A *C. botulinum* (deposited with the ATCC) is used to produce type A toxin. This strain is routinely used for production of type A botulinum toxin due to high toxin titers and the rapid onset of cell lysis (usually within 48 h).

For toxin production, cultures of the Hall A strain are grown statically in 10–20 liter volumes of toxin production medium (TPM) consisting of 2.0% NZ amine or TT (Sheffield Laboratories, Norwich, N.Y.), 1.0% yeast extract (Difco), and 0.5% dextrose, pH 7.37.4, for 5–7 days at 37° C.

To prepare essentially pure type A neurotoxin, the type A toxin complex is first purified according to the method described in the Ph.D. thesis of M. C. Goodnough (Goodnough, M. C. 1994, Characterization and stabilization of *Clostridium botulinum* toxin for medical use. Ph.D. thesis, UW-Madison, as adapted from Tse et al. 1982)

Type A neurotoxin is purified from the associated nontoxic proteins of the complex by a modification of the method of Tse et al. (1982) (Goodnough, M. C., 1994, Thesis, UW, Wisconsin). Toxin complex is recovered from the DEAE-Sephadex A50 (Sigma Chemical Co., St. Louis, Mo.), pH 5.5, column and is precipitated by addition of 39 g of solid ammonium sulfate/100 ml. The precipitated toxin complex is collected by centrifugation, dialyzed against 25 mM sodium phosphate, pH 7.9, and applied to a DEAE-Sephadex A50 column equilibrated with the same buffer. Toxin is separated from the non-toxic proteins of the complex and eluted from the column with a linear 0–0.5M sodium chloride gradient. Partially purified neurotoxin is recovered from the DEAE-Sephadex A50 column at pH 7.9 and dialyzed against 25 mM sodium phosphate, pH 7.0. The dialyzed toxin is applied to SP-Sephadex C50 (Sigma Chemical Co.) in 25 mM sodium phosphate, pH 7.0. Contaminating material does not bind to the column under these conditions. The neurotoxin is eluted with a linear 0–0.25M sodium chloride gradient. The neurotoxin can be further purified by metal affinity chromatography, gel filtration or other methods of protein chromatography.

For lyophilization, toxin samples are diluted in the excipients (stabilizing compounds) to be tested (Sigma Chemical

Co.), 0.1 ml or 0.5 ml aliquoted into 2 ml glass vials (Fisher Scientific Co., Pittsburgh, Pa.), the Teflon lined screw cap closures fastened loosely, and the samples are quickly frozen in liquid nitrogen. The frozen samples are placed into a lyophilization flask which is then immersed in liquid nitrogen. The flask is then connected to a laboratory freeze-drier (Virtis Freezmobile 12, Virtis Co., Inc., Gardiner, N.Y.). When the pressure drops below ca. 60 mTorr, the liquid nitrogen jacket is removed. Pressure is maintained at or below 30–60 mTorr and condenser temperature constant at −60° C. Samples are allowed to come to room temperature and drying continued at ambient temperature over the next 18–24 h. At that time the flask is removed and the vials tightly capped. Vials were assayed for toxicity and recovery calculated within 1–3 days (adapted from Goodnough and Johnson, 1992).

Vials of lyophilized type A neurotoxin and type A toxin complex were stored at various temperatures to investigate the effect of added excipients on the shelf-stability of the dried material. In these cases, the tightly capped vials were placed into plastic bags, sealed and stored at various temperatures (−20°, 4°, or 37° C.) and the contents assayed for toxicity at various time points. The lyophilized preparations were usually reconstituted in 1.0 ml of distilled water. The use of 0.85% saline for reconstitution gave equivalent results. The white cake dissolved immediately and was mixed by gentle inversion of the vials. The resulting solution was transparent and contained no particulates. This solution was titrated by the same method used for the prelyophilization solution. The percent recovery (calculated as number of mouse intraperitoneal lethal doses per vial after lyophilization divided by the number of mouse intraperitoneal lethal doses before lyophilization×100) represent averages of trials done in at least duplicate. The variation in independent assays was ca. ±20%.

The percent recoveries of active toxin determined within 2–3 days following lyophilization of type A toxin complex and purified type A neurotoxin in various excipient combinations are shown in Tables 1 and 2.

The primary advantages of preferred compositions of the present invention are their high percentage recovery of biologically active neurotoxin and their long-term stability (shelf life) at temperatures above 0° C. In contrast, the current commercial product has a low percentage recovery of biologically active neurotoxin and must be stored at temperatures of −10° C. or less.

TABLE 1

Effect of excipients on recovery of toxicity of *Clostridium botulinum type A toxin* complex after lyophilization of 0.1 ml of each solution.

Excipients	Starting Toxin concentration[a]	pH	% recovery[b]
sodium phosphate[c]	50, 100, 1,000	5.0, 6.0, 6.8	<10
bovine serum albumin/sodium chloride[d]	100	6.4	10
bovine serum albumin[e]	100, 1,000	6.4	88, 75
bovine serum albumin/citrate[f]	100, 1,000	5.0	>90, >90
bovine serum albumin/phosphate[g]	100, 1,000	5.5	>90, >90
bovine serum albumin/phosphate[g]	1,000	7.3	60
bovine serum albumin/phosphate[h]	1,000	6.0	>90

TABLE 1-continued

Effect of excipients on recovery of toxicity of *Clostridium botulinum type A toxin* complex after lyophilization of 0.1 ml of each solution.

Excipients	Starting Toxin concentration[a]	pH	% recovery[b]
human serum albumin[i]	100, 1,000	6.4–6.8	>90, >90
alpha-lactalbumin[j]	1,800	6.1	>78
lysozyme[j]	1,800	5.3	>78
gelatin[j]	1,800	6.3	>78
bovine serum albumin/trehalose[k]	500	5.7	>90
bovine serum albumin/sucrose[l]	325	6.6	65
bovine serum albumin/maltotriose[m]	250	7.0	>80

[a]Type A mouse lethal doses/vial before lyophilization;
[b]% recovery = (number mouse lethal doses after lyophilization/number mouse lethal doses prior to lyophilization) × 100;
[c]50 mM sodium phosphate;
[d]bovine serum albumin (5.0 mg/ml), sodium chloride (9.0 mg/ml);
[e]bovine serum albumin (9.0 mg/ml);
[f]bovine serum albumin (9.0 mg/ml), 50 mm sodium citrate;
[g]bovine serum albumin (9.0 mg/ml), 50 mm sodium phosphate;
[h]bovine serum albumin (9.0 mg/ml), 50 mm potassium phosphate;
[i]human serum albumin (9.0 mg/ml);
[j]concentration = 9.0 mg/ml;
[k]9.0 mg/ml bovine serum albumin, 100 mg/ml trehalose;
[l]9.0 mg/ml bovine serum albumin, 250 mg/ml sucrose;
[m]9.0 mg/ml bovine serum albumin, 100 mg/ml maltotriose. (adapted from Goodnough and Johnson, 1992).

TABLE 2

Recovery of activity following lyophilization of purified *Clostridium botulinum* type A neurotoxin.

Excipient combination	Starting Toxin concentration[b]	pH	% recovery[c]
bovine serum albumin	200	6.4	90
human serum albumin	1,000	6.4	90
bovine serum albumin, trehalose	500	5.7	>90
bovine serum albumin, sucrose	325	6.6	50
bovine serum albumin, maltotriose	250	7.0	>80

[a]bovine and human serum albumin concentration was 9.0 mg/ml, carbohydrate concentration was 100 mg/ml in all cases except sucrose which was 250 mg/ml;
[b]mouse intraperitoneal lethal doses/vial;
[c](number of mouse lethal doses/vial after lyophilization, number of mouse lethal doses before lyophilization) × 100.

It was found that the formulations used for lyophilization had a marked effect on the recovery of toxin. The most critical factor was the absence of sodium chloride in the solution in combination with a pH less than 7.0. In the best cases, recovery of active toxin was >90% following lyophilization.

The addition of trehalose in particular, allowed the recovery of active type A neurotoxin following lyophilization and storage at temperatures in excess of those required for storage of the commercially available type A toxin complex (37° C. versus −10° C.).

The antigenicity of various toxin preparations (containing low or high specific toxicities) was evaluated in rabbits by repetitive injection of sublethal doses of toxin simulating treatment of a focal dystonia with botulinal toxin. The samples were standardized to contain the same number of active lethal doses in order that the immune response from the rabbits could be compared. The samples with the highest specific activity were those consisting of purified type A neurotoxin (96 U/ng) while the lowest were the commercially available BOTOX®, crystalline botulinum toxin complex, samples (4.3 U/ng).

Total toxin concentration for each preparation (i.e. both active and inactive) was determined using an enzyme-linked immunosorbent assay (ELISA) specific for type A botulinal toxin. The ELISA assays performed on BOTOX®, crystalline botulinum toxin complex, and ASB indicated that BOTOX®, crystalline botulinum toxin complex, had an average specific toxicity of 4.3 U/ng and ASB had an average specific toxicity of 17.3 U/ng after reconstitution. Type A toxin complex used in these assays had a specific activity of 18 U/ng.

Serum samples were drawn on the days shown in Table 3 and titrated for the ability to neutralize 5.6 U of essentially pure type A neurotoxin. Total toxin concentration (active+inactive) in each preparation was determined by ELISA following reconstitution except in the cases of A neurotoxin and A complex which were not lyophilized.

TABLE 3

Immune response of rabbits to sub-lethal doses of type A botulinal toxin.

	A neurotoxin	A complex	Botox I*	Botox II*	ASB I*	ASB II*
Day	0	0	0	0	0	0
	29	28	21	21	21	21
	42	35	35	35	35	35
	56	56	49	49	49	46
	69	69	60 (1:1)[a]	63 (1:1)[a]	60 (no antibodies detected)	60
	88	88		77 (1:2)		67
	107	109		84 (1:4)		81
	118 (No antibodies detected)	118 (No antibodies detected)				95 (1:1)[a]
Total ng of toxin:	0.92	5.12	18.3	25.41	4.075	6.52

*Two separate animal trials labeled I and II are represented.

[a]All antibody samples were titrated against 5.6 mouse lethal doses of purified type A neurotoxin according to the following: 0.5 ml serum + 0.1 ml containing 5.6 LD_{50} type A toxin + 0.6 ml gel-phosphate, pH 6.4. The solution was incubated at room temperature for 30–60 minutes. Two mice per two fold dilution were injected intraperitoneally with 0.5 ml of serum + toxin mixture.
Dilutions which neutralized the toxin challenge are indicated in brackets. The last value in the numerical column indicates the final day of sampling. Numbers in parentheses indicate final dilution which neutralized toxin challenge.

These results show that the immune response of rabbits to botulinal toxin is dependent on concentration of the toxin (active+inactive) injected as well as the number of times the animal is exposed to that concentration. From these results it follows that the higher the specific activity of the lyophilized/reconstituted toxin product, the less antigenic material the patient is exposed to and the smaller the chances of patients developing neutralizing antibodies. Thus, it is advantageous to have pharmaceutical compositions of lyophilized essentially pure neurotoxin which permit the recovery of a high percentage of the starting activity and contain trehalose for storage of the dried product without degradation at temperatures above that of the currently available product are shown.

It will be apparent to those skilled in the art that a number of modifications and changes can be made without departing from the spirit and scope of the present invention. Therefore, it is intended that the invention be limited only by the claims.

We claim:

1. A pharmaceutical composition consisting essentially of:

(a) isolated, essentially pure type A botulinum neurotoxin;

(b) serum albumin; and

(c) an effective amount of trehalose which stabilizes the neurotoxin and improves the shelf life of composition so that it is stable at temperatures up to about 37° C.

2. A composition of claim 1 in which the botulinum neurotoxin has specific toxicity of about 80 U/ng to about 96 U/ng.

3. A lyophilized pharmaceutical composition of type A botulinum neurotoxin which is stable for up to four months at about 37° C. without the neurotoxin losing its potency, said composition consisting essentially of pure type A botulinum neurotoxin and an effective amount of trehalose to stabilize the neurotoxin.

* * * * *

XP-000982177

Botulinum Toxin A for Hyperkinetic Facial Lines: Results of a Double-Blind, Placebo-Controlled Study

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Previous work on patients with muscular dystonia has shown that small intramuscular doses of botulinum toxin A eliminated hyperkinetic facial lines for approximately 6 months. The purpose of this study was to determine the efficacy of botulinum toxin A injections in eliminating facial wrinkles in aesthetic surgery patients who do not have muscular dystonia. Eleven healthy subjects were studied in a double-blind fashion. On both sides of the face, 0.2 cc of either normal saline or botulinum toxin A was injected into the forehead or into the periorbital wrinkles (crow's feet). Documentation of results was made by photographs taken of the patients during repose and during facial animation before and after injection. Assessment of facial wrinkles was done from a grading system in which the patient and the facial plastic surgeon were asked to judge the severity of the wrinkles on a scale from 0 to 3, with 0 reflecting no facial wrinkles and 3 reflecting severe facial wrinkling.

Nine of 11 subjects injected with botulinum toxin A noted a significant improvement in the severity of their facial wrinkles in comparison with the side of the face injected with saline, with a rating improvement of 2 points. Two of 11 subjects noted a moderate improvement, with a rating improvement of 1 point. No patient injected with saline reported an improvement in the severity of the facial wrinkles on the control side. There were no serious complications. Botulinum toxin A is an efficacious method of nonsurgically eliminating facial wrinkles and may play a role in the cosmetic enhancement of the aging face. (*Plast. Reconstr. Surg.* 94: 94, 1994.)

It is well known that hyperfunctional facial lines are the result of pull on the skin by the underlying facial mimetic musculature.[1] Facial wrinkles need to be differentiated from dynamic facial creases. In contradistinction to a hyperfunctional facial line, a facial wrinkle is caused by laxity intrinsic to the skin that is the result of age-induced changes in collagen of the dermis. Specifically, hyperkinetic lines formed by the corrugator supercilii, frontalis, and lateral aspects of orbicularis oculi muscles result in glabellar frown lines, deep forehead wrinkles, and crow's feet, respectively. There are numerous surgical procedures designed to eliminate or attenuate these hyperkinetic facial lines on the aging face, including rhytidectomy, liposuction, brow lift, dermabrasion, chemical peel, and collagen injections.[2,3] Rarely have any of these techniques available for eliminating facial wrinkles been scrutinized in a double-blind, placebo-controlled manner. Previous work with patients suffering from muscular dystonia has shown that small intramuscular dosages of botulinum toxin A eliminate hyperkinetic facial lines for approximately 6 months.[1] The purpose of this study was to determine the efficacy of botulinum toxin A injections in a double-blind, placebo-controlled fashion in eliminating hyperfunctional facial lines in healthy aesthetic surgical patients.

Background and Pharmacology

The bacterium *Clostridium botulinum* produces eight serologically distinct toxins. The toxins exert their effects at the neuromuscular

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junction by inhibiting the release of acetylcholine. The result is a flaccid paralysis. There are three steps involved in the toxin-mediated paralysis: binding, internalization, and inhibition of neurotransmitter release. The binding is selective and saturable. It is the release of vesicle-bound acetylcholine that is inhibited by the toxin.

One international unit (IU) is defined as the LD_{50} in mice. The LD_{50} in humans is estimated to be approximately 2730 IU.[3] The toxin is shipped freeze dried in 100-IU vials. When used clinically in this study, the toxin is reconstituted with normal saline to a concentration of 2.5 to 5 IU/ml. The toxin takes 3 to 4 days to have a noticeable effect and lasts approximately 4 to 6 months.

Injection Technique

Toxin was freshly diluted to a concentration of 2.5 or 5 IU/ml and injected by means of a monopolar hollow-bore Teflon-coated electromyography needle which was connected to an EMG recorder. By a technique previously described, the needle was placed through the skin overlying the exaggerated facial lines.[1,4] With the needle in place, EMG readings commenced, and the patient was instructed to accentuate the lines with a squint or a frown. The needle was moved to the electrically most active portion of the muscle, and the toxin was then injected. No massaging of the site took place so as to keep the diffusion of the material to a minimum.

Patients and Methods

A total of 12 patients were included in the study. All patients were first screened by a facial plastic surgeon (Keen or Aviv). A thorough review of medical history, medications, and prior facial plastic surgery was obtained. Patients with contraindications to botulinum toxin A injections, such as patients with Eaton Lambert syndrome, patients with known hypersensitivity to the toxin,[5] and patients who could not complete the protocol, were not included in the study. Of the 12 patients enrolled in the double-blind trial, 1 was eliminated from the study because of failure to return for follow-up photographs and assessment. Of the 11 remaining patients, injections were performed in 9 patients with hyperfunctional forehead lines and in 2 with prominent crow's feet. Forehead injections included 8 injection sites with 10 units on one hemiforehead. Crow's feet injections were at 2 separate sites utilizing 5 units on the tested side. The ages ranged from 32 to 62 years, with a mean age of 42.8 years. There were 7 females and 4 males. Photographs of the injection site both at rest and during active wrinkling were taken to document the preinjection appearance. All photographs were standardized by using the same camera, lens system, flash system, and film. Photographs were obtained at 3 ft with a 105-mm lens oriented vertically utilizing Ekta Chrome 400 slide film and a ring flash. A set of close-up photographs at 2 ft with the camera oriented horizontally also were obtained. These photographs were repeated at 2 and 6 weeks after injection. All patients were followed up for a minimum of 1 year. Sites injected included forehead lines and crow's feet (Table I).

Subjects were studied in a double-blind fashion. On both sides of the face, 0.2 cc of either normal saline or botulinum toxin A was injected into either the periorbital wrinkles (crow's feet) or the forehead frown lines. The

TABLE I
Patient Data

				Rating Before Injection		Rating After Injection		Net Improvement (6 wks)	
Patient	Age (years)	Sex	Region	Rest	Wrinkles	Rest	Wrinkles	Rest	Wrinkles
JB	32	M	Forehead	1	2	0	0	+1	+2
BB	48	F	Forehead	2	3	1	2	+1	+1
CC	40	F	Forehead	2	3	Did not return for follow-up			
MK	36	M	Crow's feet	1	2	0	0	+1	+2
LL	32	F	Forehead	2	3	0	1	+2	+2
DO	62	F	Crow's feet	2	3	2	2	0	+1
CR	37	F	Forehead	2	3	0	0	+2	+3
IR	47	F	Forehead	2	3	0	0	+2	+3
CT	44	M	Forehead	2	3	1	1	+1	+2
AV	42	F	Forehead	3	3	0	1	+3	+2
EE	50	F	Forehead	2	3	1	2	+1	+1
WB	44	M	Forehead	2	3	0	1	+2	+2

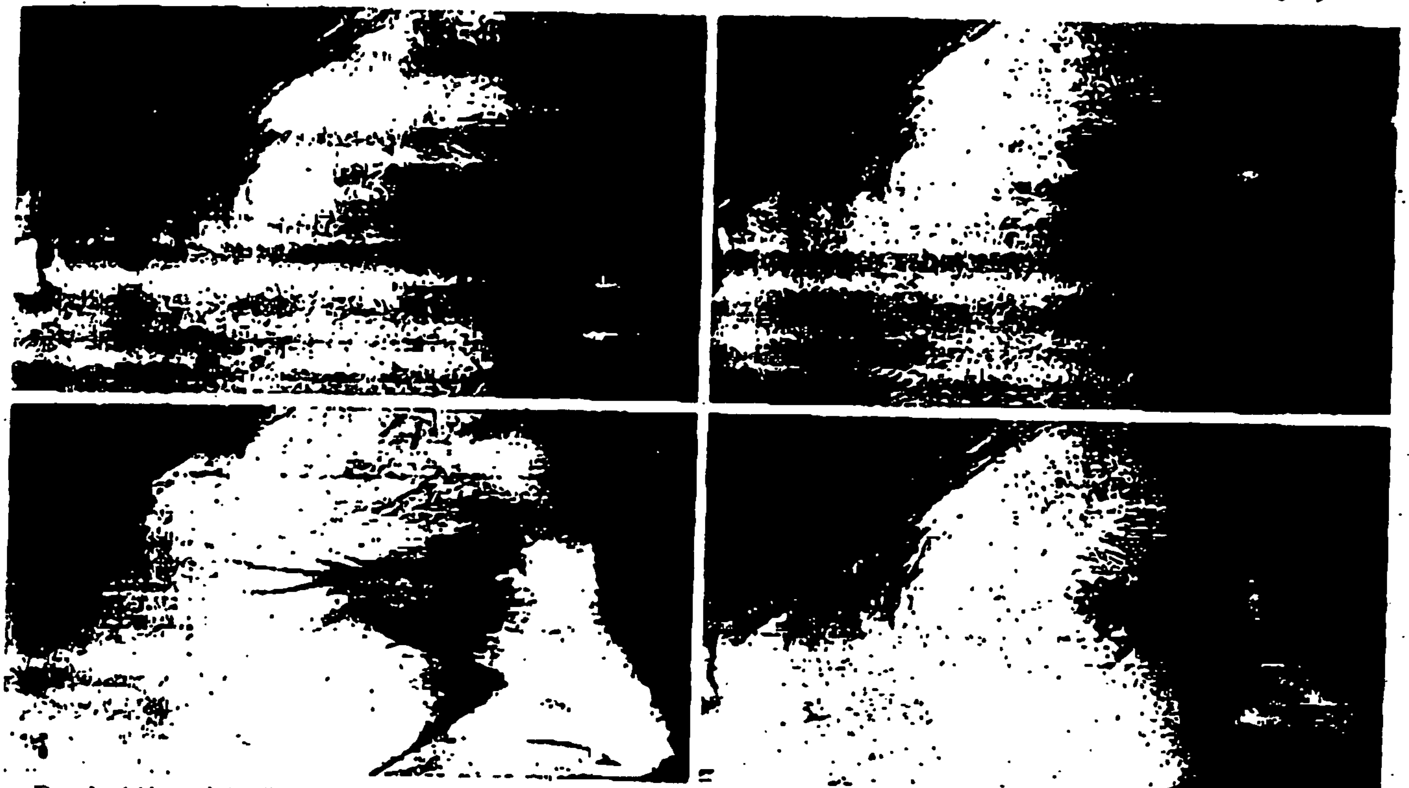

FIG. 1. (*Above, left*) Close-up of crow's feet at rest before injection, right eye. (*Above, right*) Same patient 2 weeks after botulinum toxin injection to lateral portion of orbicularis oculi at rest, right eye. (*Below, left*) Same patient before botulinum toxin injection squinting, right eye. (*Below, right*) Same patient with crow's feet attenuated by botulinum toxin injection 2 weeks after injection, squinting, right eye.

patients were asked to rate their wrinkles before injection and 2 weeks after receiving bilateral placebo-controlled injections. Patients had access to their prior responses when grading their wrinkles. Photographs taken at both visits were randomized and presented to the senior author for judging. The data were analyzed by paired *t* analysis.

A total of 5 units was utilized for the crow's feet, and 10 units was utilized for the forehead injections. After 6 weeks, patients were offered an injection of toxin on the control side.

Prior to the injections, all patients were asked to assess their facial wrinkles by looking in a mirror and grading the severity of the wrinkles. The rating system devised by our group is based on a scale of 0 to 3, with 0 reflecting no facial wrinkles, 1 signifying mild facial wrinkles, 2 denoting moderate facial wrinkles, and 3 representing severe facial wrinkling (Table II).

These assessments were made both in repose and during animation. At this time, no grading was performed by the surgeons, and no cues were given by the surgeons. The surgeons graded the wrinkles from slide projections of the photographs at a separate sitting approximately 2 months after the testing was completed. The surgeons did not have access to the patients' grades, and they were blinded.

Results

There was no significant change in facial wrinkle assessment before and after injections of saline, as measured by both patients and examiners. When measuring the severity of facial wrinkles in patients injected with toxin, patients in repose reported a mean reduction in wrinkles of 1.3 rating points (out of 4). The surgeons rated the mean reduction to be 1.5 points. This was a significant improvement, with paired *t* analysis revealing $p < 0.01$. When patients were asked to contract the injected muscles, the results were even more impressive. A profound improvement was n ted, with the patients reporting a mean improvement of 2.0 full rating points and the surgeons concurring

TABLE II

Facial Wrinkle Grading System

0 = no wrinkles
1 = mild wrinkles
2 = moderate wrinkles
3 = severe wrinkles

with a 1.9-point mean improvement. Again, this was statistically significant according to paired *t* analysis ($p < 0.01$).

All subjects requested a botulinum toxin A injection to even off the results. There were no serious complications. Three of 11 patients thought the injection was painful, and 2 of the 11 patients reported that their eyebrow had dropped slightly. One other patient reported that the shape of her eyebrow had changed slightly, and that same patient stated that her forehead felt "heavy" on the toxin-injected side. All patients were pleased with the change in their appearance, and 10 of 11 patients returned for further injections when the material wore off.

Discussion

Hyperfunctional facial lines involving the forehead and the lateral orbital region are common cosmetic deformities. These excessively prominent lines can be interpreted as anger, anxiety, fear, fatigue, melancholia, and aging. In the past, these lines have been treated by surgical excision, yielding unsightly scars, or injected with collagen, silicone, or even the patient's own fat in an effort to balloon out the skin and flatten the folds.[1-3] Face lifts and brow/forehead lifts are surgical endeavors that only partially improve these wrinkles and at a significant cost in terms of scarring and healing. These treatments do not adequately address the fact that these face lines are functional and are related to the pull of the underlying mimetic facial musculature. Just as a patient with a facial paralysis does not have a line on his or her face, the flaccid paralysis that is induced by small quantities of botulinum toxin A effectively eliminates these wrinkles. The toxin weakens the mimetic muscles and thereby physiologi-

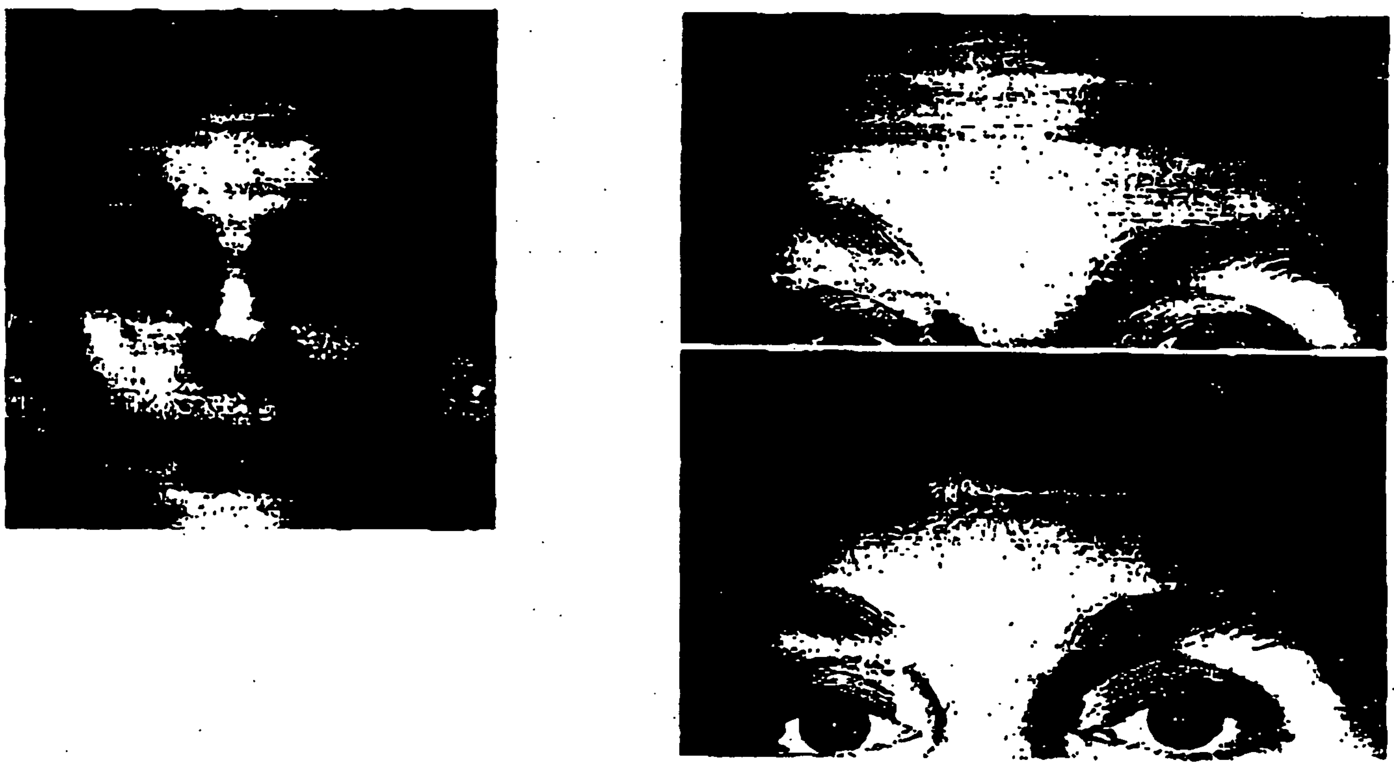

FIG. 2. (*Above, left*) Full-face view of double-blind forehead injection at 2 weeks. Note elimination of wrinkles on the botulinum toxin side, elevating eyebrow. (*Above, right*) Close-up, elevated eyebrow. (*Below, right*) Same patient 2 weeks after having control side injected with toxin to balance off aesthetic appearance, elevating other eyebrow. Note lack of wrinkles.

FIG. 3. (*Above*) Before injections, elevating forehead. (*Below*) Same patient after botulinum toxin injections laterally and saline in the midline, 2 weeks after injection, elevating forehead.

cally lessens the wrinkles that are the product of the muscular contraction.[1,4–8] Not all facial wrinkles are caused by the tension of the underlying mimetic muscles. Laxity of the skin that is related to the age-induced changes in dermal collagen also can cause facial wrinkles. Indeed, our poorest result was in our eldest patient, in whom these factors played a significant role in the origin of the wrinkles. The toxin can be injected easily on an ambulatory basis with minimal discomfort. By utilizing EMG control with small volumes of concentrated solution, one can precisely localize the injection. This can be accomplished with minimal side effects.

Graded weaknesses can be achieved by utilizing low dosages initially and then repeating the injections, if necessary, to achieve the desired effect. If too much weakness is achieved, the toxin gradually wears off in 4 to 6 months. By starting with a low initial dose, undesirable side effects can be avoided. The injections need to be repeated every 4 to 6 months in order to sustain the results. This may be considered an inconvenient drawback to the procedure, but this represents an improvement over collagen, which lasts only 2 to 3 months, and to silicone, which is currently not legally available. Full function returns without any long-term sequelae. Side effects were limited to occasional brow asymmetry and the resultant puffiness of the upper lids that occurred secondary to the brow ptosis. This was addressed by not injecting any wrinkles within 1 cm of the eyebrow. There were no complaints of adverse or limited facial expression in the sites we injected, nor was there any ptosis or ectropion of the eyelids. The results were present for approximately 4 to 6 months.

Antibodies to the botulinum toxin A have been described in patients receiving much larger dosages for longer periods of time.[5,6] The antibodies can render the toxin noneffective but do not harm the patient. No antibody production has been described in patients receiving botulinum toxin A for blepharospasm.[7–9] The dose used is two to five times the dose necessary for aesthetic indications. Whenever one injects a potentially dangerous drug into normal individuals, one must be certain that there are no long-term deleterious side effects. It should be noted that there have been no long-term adverse effects with the use of botulinum toxin A for blepharospasm and Meige's disease.[7–9] Muscle biopsies taken from patients after repetitive injections have failed to show any long-term evidence of permanent degeneration or atrophy, and these patients have received dosages that were two to five times the dosages used for aesthetic improvement of their wrinkles and have been studied for over 7 years.[10,11] Local or transient effects of botulinum toxin A have shown that excessive neuronal sprouting and muscle fiber atrophy occur. These changes are not permanent, nor are they clinically significant.[12] Aside from pain—the injection is painful—and a slight droop to the eyebrows, we did not report any other adverse reactions or side effects. Clearly, this is a short-term method of eliminating facial wrinkles and will need to be repeated two or three times a year in order to achieve a lasting result. A word of caution is necessary to the surgeon who has not had experience with this drug. Although we are recommending relatively low doses of the toxin, there is a very real danger of injecting too much toxin in the wrong areas of the face and achieving an unsightly facial palsy. This is so

because the toxin takes 3 to 5 days to give an effect, and there is no way to monitor exactly what wrinkles will be eliminated with each specific injection. We recommend the EMG injection technique as a way to ensure the correct placement of the toxin. A week after the original injections, a second "touch-up" shot may be necessary. Once a surgeon has experience with the injection technique, the control afforded by the EMG electrode may not be necessary. We are presently studying this by injecting one side with the EMG electrode needle control and the other side without it. This will be the subject of another report. Since everyone's reaction to the drug is different, it is vitally important to underdose at the first session in order to ascertain the individual patient's reaction to the medication. After injection, one must wait for the toxin to be metabolized, since there is no medication that can readily reverse the metabolic effect.

A larger study is currently being implemented to further define the aesthetic surgical indications of botulinum toxin A in eliminating facial lines. This study will include approximately 200 patients from two different cities (New York and Los Angeles) who will be injected in five different sites (forehead, frown lines, crow's feet, nasolabial folds, and platysma bands), photographed, and followed for at least 1 year. In this efficacy study, we did not attempt to define the optimal dosage, duration of effects, necessity for the use of EMG, or extrapolation of the technique to other sites. The parallel study is under way to answer these and other questions. The sole purpose of this study was to prove the efficacy of utilizing botulinum toxin A for elimination of hyperfunctional facial lines. Preliminary results indicate that the toxin seems to be most beneficial in the 30- to 50-year-old age group and most effective in the upper third of the face (crow's feet and forehead frown lines). These results will be the subject of another report.

Conclusions

We conclude that botulinum toxin A is a safe and efficacious method of nonsurgically eliminating facial wrinkles in the aesthetic surgical patient for a period of 4 to 6 months. Botulinum toxin A may play an increasingly important role as an adjuvant treatment in the cosmetic surgical enhancement of the upper third of the aging face. Botulinum toxin A injections address the need for a short-term reversible therapy to "touch up" the aging face.

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References

1. Blitzer, A., Brin, M. F., Keen, M. S., and Aviv, J. E. The use of botulinum toxin for the treatment of hyperfunction lines of the face. *Arch. Otolaryngol. Head Neck Surg.* 119: 1018, 1993.
2. Newman, J., Dolsky, R. L., and Nguyen, A. Facial profileplasty by liposuction extraction. *Otolaryngol. Head Neck Surg.* 93: 718, 1985.
3. Pitanguy, I. Indications for treatment of frontal and glabellar wrinkles in an analysis of 3404 consecutive cases of rhytidectomy. *Plast. Reconstr. Surg.* 67: 157, 1981.
4. Brin, M. F., Fahn, S., Moskowitz, C., Friedman, A., Shale, H. M., Greene, P. E., Blitzer, A., List, I., Lange, D., and Lovelace, R. E. Localize injections of botulinum toxin for the treatment of focal dystonias and hemifacial spasms. *Adv. Neurol.* 50: 599, 1988.
5. Lange, D. H., Rubin, M., Greene, P., et al. Distal effects of locally injected botulinum toxin: Incidences and effects. *Adv. Neurol.* 50: 609, 1988.
6. Savino, P. J., Sergot, R. C., Bosley, T. M., et al. Hemifacial spasm treated with botulinum A toxin injection. *Arch. Ophthalmol.* 103: 1305, 1985.
7. Tsoy, E. A., Buckley, E. G., and Dutton, J. J. Treatment of blepharospasm with botulinum toxin. *Am. J. Ophthalmol.* 99: 176, 1985.
8. Scott, A. B., Kennedy, R. A., and Stubbs, H. A. Botulinum A toxin injection as a treatment for blepharospasm. *Arch. Ophthalmol.* 103: 347, 1985.
9. Mauriello, J. A., Jr. Blepharospasm, Meige's syndrome, and hemifacial spasm: Treatment with botulinum toxin. *Neurology* 35: 1499, 1985.
10. Borodic, G. E., and Ferrante, R. Orbicularis Muscle Histology after Repetitive Injections of Botulinum A Toxin. Presented at the Annual Meeting of the American Academy of Ophthalmology, Atlanta, Georgia, 1990.
11. Scott, A. B., Kennedy, R. A., and Stubbs, H. A. Botulinum Toxin. Presented at the Annual Meeting of the American Academy of Ophthalmology, Atlanta, Georgia, 1990.
12. Borodic, G. E., Cheney, M., and McKenna, M. Contralateral injections of botulinum A toxin for the treatment of hemifacial spasm to achieve increased facial symmetry. *Plast. Reconstr. Surg.* 90: 972, 1992.

11
Cosmetic Uses of Botulinum A Exotoxin

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I. INTRODUCTION

The recent successful use of botulinum A exotoxin (BTX-A) for cosmetic applications has encouraged many clinicians to adopt its application. It is somewhat paradoxical that botulinum A exotoxin (BTX-A), considered one of the most toxic substance known to humanity (1), is one of the safest and most effective treatments for the management of neuromuscular disorders characterized by muscle hyperactivity (2). Its introduction to cosmetic applications was based on observations that facial lines and wrinkles were dramatically decreased in patients treated with BTX-A for essential blepharospasm (3). Since many lines and wrinkles are due to muscular contractions, weakening or paralyzing relevant muscle groups with the toxin produces a smoother skin appearance. BTX-A produces its effects on muscles by causing a reversible reduction in muscle contractions (4).

BTX-A, commonly known for its infamous connection to lethal food poisoning (botulism), was first tested in monkeys more than 20 years ago to treat strabismus (5). It was shown that injection of the toxin into the overacting extraocular muscle produced a reversible local muscular paralysis, resulting in an improvement in strabismus. The doses of BTX-A used to reverse strabismus in the monkey were not systemically toxic, and they did not produce serious side effects. The data from the animal studies were so encouraging that the same researcher, Scott (4,6,7), administered BTX-A to humans with strabismus. In the clinical studies, BTX-A proved

extremely effective and is now accepted treatment for some forms of strabismus.

Since the studies by Scott, the number of clinical applications of BTX-A has rapidly expanded. A review by Brin suggests that BTX-A should be effective "wherever muscles overcontract" (8). Currently, there is a list of more than 20 muscular diseases (dystonic and nondystonic spasms) in which BTX-A has proved to be a useful treatment in humans (8).

The focus of this chapter is on the application of BTX-A as a cosmetic agent to reverse facial lines and wrinkles. BTX-A therapy offers the clinician and patient many benefits in terms of effectiveness, safety, and ease of administration (9–13). In addition, the toxin acts in a selective and reversible manner. Thus, there is a relatively low incidence of side effects, all of which are limited due to the reversible nature of the compound. For these reasons, the authors recommend BTX-A as a safe and effective alternative to certain types of surgery and tissue augmentation techniques.

II. BACKGROUND

A. Subtypes of BTX-A

Botulinum neurotoxin (BTX) is produced by an anaerobic bacterium, Clostridium botulinum. This bacterium produces seven different serological types of BTX (A to G) of which BTX-A is the most potent in producing muscular paralysis in humans (5). BTX-A is the only commercially available botulinum neurotoxin to date. However, other serotypes are currently being investigated (i.e., BTX-B and BTX-F) as alternative treatments for those individuals who develop BTX-A antibodies and become nonresponsive to BTX-A treatment (see immunogenicity section below).

B. Mechanism of Action

1. Effects at Cholinergic Nerve Terminals

BTX-A produces paralysis by binding to presynaptic cholinergic nerve terminals and blocking the release of acetylcholine at the neuromuscular junction (NMJ) (14). A four-step process described by Simpson illustrates these actions (15). First, the free end of the neurotoxin molecule binds to the cholinergic receptors located on the presynaptic neuron of the NMJ (16,17); this step takes approximately 30 minutes (18). In the second step, which is energy-dependent, the internalization of the cell membrane with the bound toxin takes place (19). In the third stage, the toxin is released into the cytoplasm. In the final, fourth, step, BTX-A acts as a zinc-depen-

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2. Histological Effects

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3. Restorative Processes

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dent metalloendoprotease to deactivate specific components of the neuroexocytic mechanism by enzymatic cleavage (15,20,21).

This unique mechanism of action makes BTX-A very specific and allows a very small amount of toxin to produce a significant effect. Therapeutic doses of the compound are extremely small (1), allowing treatment to be very specific and making systemic side effects unlikely.

2. Histological Effects

The results of injection of BTX-A have been studied in both human tissue (i.e., orbicularis oculi muscle removed from patients during blepharoplasty) and animal tissue treated with the toxin (22). In the first two weeks after treatment with BTX-A, the injected muscle begins to atrophy and the size of the individual muscle fibers change. The atrophy continues for at least 4 weeks postinjection, at which point it stabilizes. Concomitant with these changes, alterations in acetylcholinesterase (AChase) activity can be seen. Normally, AChase is localized to NMJ, but exposure to BTX-A results in a spreading of the AChase over most of the sarcolemma. These effects are reversed after 3–6 months when the enzyme activity is again localized at NMJ.

3. Restorative Processes

Although the exposure to BTX-A produces permanent inactivation of exposed cholinergic terminals, the return of normal muscle function occurs through such processes as turnover and repair, axonal sprouting, the production of new NMJ, and the absorption of senescent or dysfunctional axon terminals. Paradoxically, BTX-A accelerates repair processes following its toxic effects at the NMJ. Within 10 days of exposure to BTX-A, new unmyelinated axonal sprouts at both pre- and postsynaptic terminals can be observed (23). They subsequently join with new but smaller endplates formed at the same time along the damaged NMJ. The newly formed NMJ connections are not as well organized as the original NMJ (single NMJ may be innervated by more than one sprout and vice versa), but after a period of reprogramming and reorganizing, the new motor endplates are activated and muscle function is restored. The restorative process is fairly consistent in response to BTX-A, and, within approximately 3–6 months, power is restored to the muscle. Other processes, such as resorption and remodeling, however, take more time, and histologic changes may be observed as much as 3 years after BTX-A injection (24,25). These changes, however, are of no major clinical consequence since the muscles are normal in terms of response and power.

4. Onset and Duration of Clinical Effects

The timing of the therapeutic action of BTX-A is related to the course of events at the NMJ. At therapeutic doses, initial effects of the toxin usually occur within 2–3 days postinjection. Maximal weakness, however, occurs later, at approximately 1–2 weeks postinjection, when the treated muscles atrophy. In general, the smaller the dose of BTX-A, the longer it takes to see clinical effects, probably due to a decrease in the amount of toxin acting at the NMJ.

The clinician should expect that weakness in the treated muscles should last approximately 3–6 months. The long duration of the action of BTX-A is probably related to the mechanisms involved in the recovery processes (e.g., sprouting). The duration of the restorative processes varies among individuals; in our experience, we have found that repeated BTX-A treatments may produce therapeutic effects lasting for as long as 12 months.

III. TECHNOLOGICAL CONSIDERATIONS

A. Sources of BTX-A

There are currently two commercially available sources of BTX-A, Botox® and Dysport®. The authors' clinical experience is only with Botox, and all references in this chapter refer to Botox unless otherwise stated. However, the clinician should be aware that there are significant clinical differences between the two sources. In clinical use, Botox is 3 to 4 times stronger (in mouse units) than Dysport, and the dose must be adjusted accordingly. As an example, a 25-unit dose of Botox would be equivalent to a 75- to 100-unit dose of Dysport (10).

B. Availability

Botox is a sterile, lyophilized form of botulinum toxin type A produced from a culture of the Hall strain of C. botulinum grown in a medium containing A–Z amine and yeast extract. It is purified from the culture solution by a series of acid precipitations to a crystalline complex consisting of a high-molecular-weight toxin protein and an associated hemagglutinin protein. The crystalline complex is redissolved in a solution containing saline and albumin and sterile filtered (0.2 microns) prior to lyophilization.

Botox is available in a vial and needs to be reconstituted with sterile, nonpreserved saline prior to intramuscular injection (see below for dilu-

Botox® is a registered trademark of Allergan, Inc.
Dysport® is a registered trademark of Speywood Pharmaceuticals.

tion methods). Each vial contains 1
0.5 milligrams of human albumin a
in a sterile, lyophilized form withou
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units) (26–28).

Dysport is also available in
hemagglutinin complex. It is pro
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contains 500 mouse units, althoug
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adjusted accordingly.

C. Dilution

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tion methods). Each vial contains 100 units of C. botulinum toxin type A, 0.5 milligrams of human albumin and 0.9 milligrams of sodium chloride in a sterile, lyophilized form without a preservative. One unit corresponds to the calculated median lethal intraperitoneal dose (LD_{50}) in mice of the reconstituted Botox that is injected. The 100 units of Botox in each vial are significantly below the LD_{50} dose in a person weighing 70 kg (2500–3000 units) (26–28).

Dysport is also available in sterile lyophilized form of the toxin/hemagglutinin complex. It is produced by column-based purification rather than by the precipitation technique used for Botox. The Dysport vial contains 500 mouse units, although, as indicated earlier, these units are less effective than the Botox mouse unit in humans, and the dose must be adjusted accordingly.

C. Dilution

According to the manufacturer's instructions, both Botox and Dysport should be diluted with preservative-free saline 0.9%. However, Garcia & Fulton suggest that dilution of Botox with saline and preservative (0.9% benzyl alcohol) is equally effective (29). Additional data is needed to verify the effects of benzyl alcohol on efficacy and interactions with the toxin.

The solution of BTX-A toxin is vulnerable to surface denaturation caused by bubbles associated with shaking. Therefore, care should be taken during the various procedures (e.g., dilution and filling the syringe) not to agitate the solution. During dilution, for example, the saline should be gently introduced into the vacuum of the toxin vial to prevent foaming.

The dilution of the toxin (i.e., dose per unit volume) varies with the area being treated. In general, a higher concentration (50 or 100 units/ml) allows for more accurate placement and therefore greater duration of effect and less side effects. Lower dilutions (5–25 units/ml) will encourage the spread of the toxin, perhaps creating a smoother effect. For cosmetic use, there are two schools of thought concerning the dilution and volume for optimal effects. The Fulton method involves the administration of large volumes of low-dose toxin to smooth crow's feet and brow area (29). This method works by weakening but not paralyzing underlying facial muscles. In contrast, an alternative method used by the authors is to apply low-volume, concentrated toxin to paralyze specific muscles (e.g., as in the glabellar area) (9). These two techniques are not mutually exclusive but should be regarded as different approaches which can be modified to individualize treatment with BTX-A.

There is evidence that reconstituted Botox loses significant toxicity 12 hours after dilution (30), although others report that the toxin is clinically effective for longer periods, possibly as long as one month (29). According

to the manufacturer's instructions, the reconstituted vial should be used within 4 hours; consequently, it is recommended that patients should be "batched" so that multiple doses can be given from a single reconstituted vial of toxin.

IV. CLINICAL CONSIDERATIONS

There are very few technical considerations in the use of BTX-A for cosmetic purposes, mainly because the therapeutic dose is low and the effects are localized and reversible. In addition, because the toxin has a high specificity for the receptor (high affinity and irreversible binding), administration of the toxin by intramuscular injection produces localized effects with minimal spreading. Furthermore, side effects associated with the toxin are reversible within a relatively short period of time.

A. Immunogenic Properties

There are no reported cases of the development of treatment-related antibodies associated with therapeutic doses of BTX-A used in cosmetic applications (31). However, the clinician should be aware that BTX-A does have immunogenic properties; conditions likely to increase the risk of antibody formation should be avoided.

Currently, investigators are trying to develop more accurate methods of using immunological assays to determine the quantity of BTX-A antibodies in humans. Thus far, methods are not accurate or sensitive enough to detect small amounts of antibodies in humans exposed to BTX-A. An enzyme-linked immunosorbent assay (ELISA) appears somewhat promising but has yet to demonstrate accuracy in predicting clinical resistance to BTX-A treatment (32–34). At present, the mouse LD_{50} assay remains the benchmark.

The incidence of treatment resistance to BTX-A usually varies with the amount of exposure to the toxin. In neurologic patients, it is estimated that one-third of all treatment failures may be a result of the development of antibodies (35). Patients treated for cervical dystonia with high doses of toxin (100–1200 units/session) have an incidence of 3%–5% for antibody formation (32,36–38). In contrast, patients treated with relatively low doses (e.g., patients treated with doses ranging from 12.5 to 50.0 units per eye for blepharospasm or 10–50 units/session for aesthetic purposes) have not been reported to develop any antibodies. In our 13 years of experience with more than 10,000 injection sessions involving BTX-A, we have not seen any cases of proven antibody-mediated treatment resistance. Thus, to date, there appears to be little risk of antibody development in patients treated with BTX-A doses below 100 units/session.

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B. Use of Electromyograph

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V. GENERAL PROCEDUR

A. Pretreatment Considera

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B. Preparation of Facial A

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It is important for the treating clinician to be aware of the clinical situations where the development of antibodies are most likely to occur. These include: patients injected with toxin doses greater than 100 units/session; patients receiving booster injections within 30 days of the initial BTX-A injection (7); and injections of toxin into systemic circulation (7). In any of these instances, alternative treatments with less potent forms of BTX (i.e., BTX-B or BTX-F) may be effective, as these toxins are not cross-reactive (39,40).

B. Use of Electromyography (EMG)

Since the major effect of BTX-A is muscular, some clinicians use electromyography (EMG) to increase the accuracy of injections (13,41,42). The EMG will locate active muscles accurately. When treating Bell's palsy, for example, EMG will provide valuable information on helping to assess injection sites for the reversal of asymmetry. However, the technique is costly and makes the procedure more cumbersome. In our clinic and in others, EMG is not routinely used (10,29,43). It is useful to assist in the learning process, to find functional muscles in a partially weakened area (touchups), and for small, accurate doses.

V. GENERAL PROCEDURES AND EVALUATIONS

A. Pretreatment Considerations

In the preassessment period, the clinician should determine the areas requiring treatment with BTX-A, the location and number of injection sites, and the desired muscular effect of the toxin on relevant facial regions. The actions of the toxin can range from a slight weakening to complete paralysis of the injected muscles. The clinical effect will be determined by toxin dilution, total solution volume, and the number and location of injections sites. Where appropriate, we have made recommendations for these variables based on our experience and that of other clinicians. Of utmost importance is the consideration of individual differences in the underlying anatomy in muscle function, and in the scope of the problem.

B. Preparation of Facial Areas

Prior to injection, the cutaneous areas should be cleansed with an alcohol swab. We also recommend that the area to be treated be chilled with an icepack before and after treatment, as some patients complain of pain and stinging. A eutectic mixture of local anesthetic (EMLA) can also be used prior to injection.

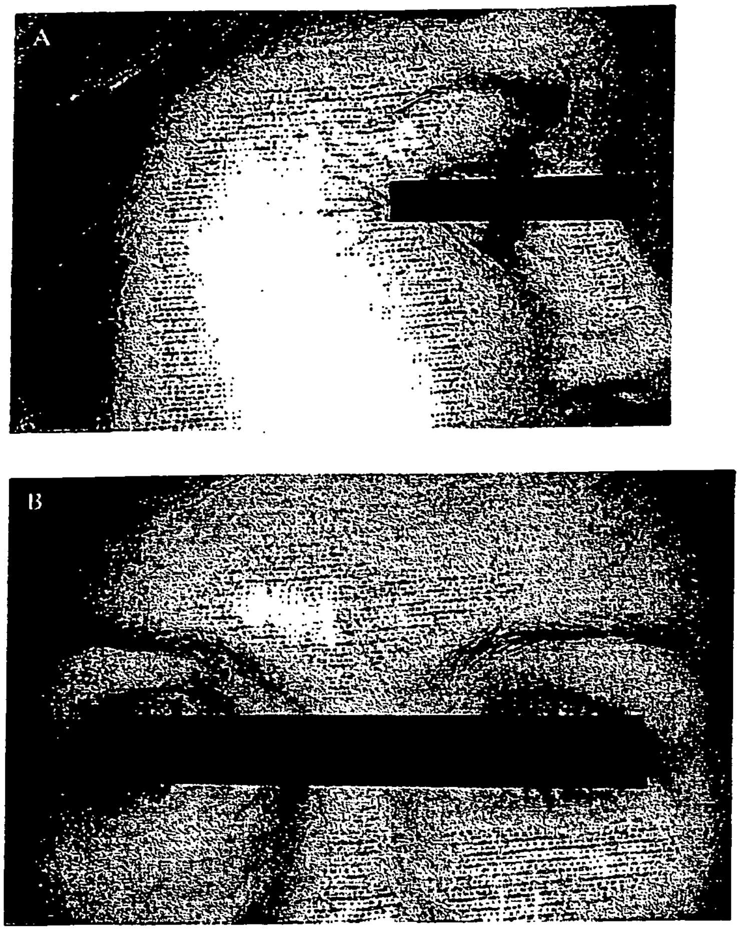

Figure 1 A and B.

C. Evaluation of Clinical Outcome

In our experience, assessment by the subject and a trained observer provide the most optimal information about the effects of BTX-A therapy. The value of photographs is limited due to the number of variables involved in photography (e.g., lighting, type of film, camera, etc.). However, still photographs, although not ideal, may be used to demonstrate the inability to move specific facial muscles

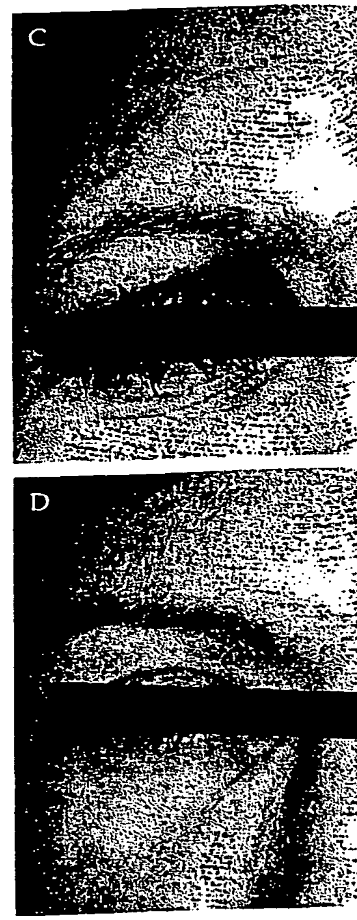

Figure 1 Individual at rest, bef
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D. Charting Methods

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Chart information should inc

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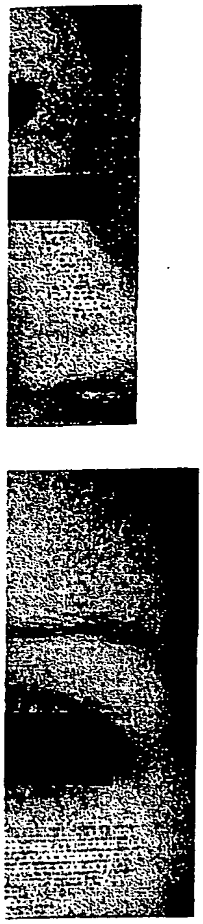
2. Dosage (in units) t



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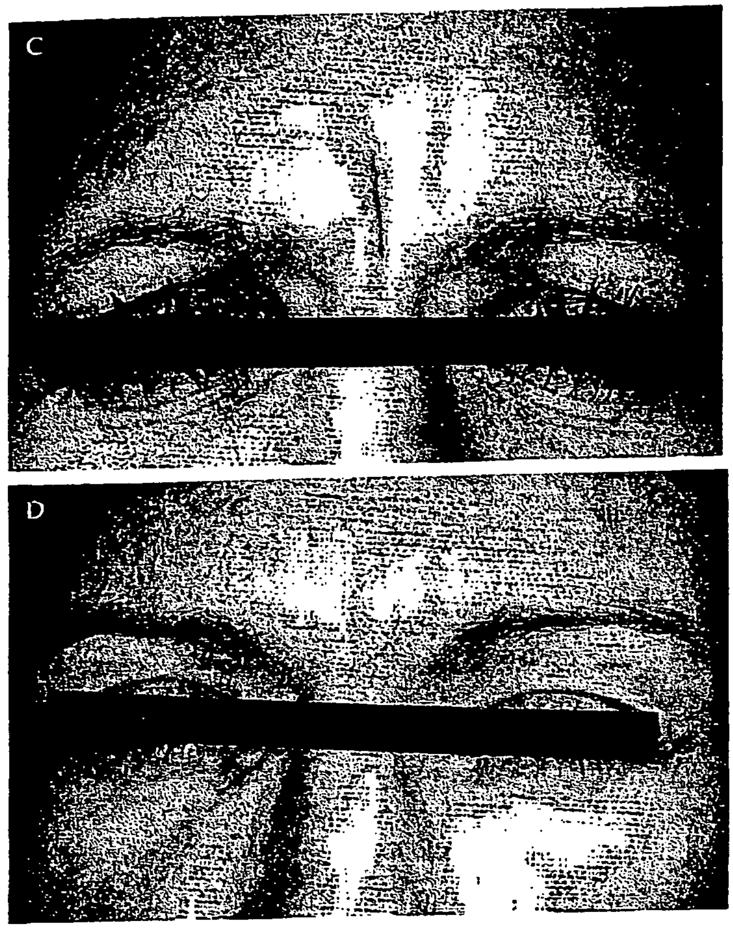

Figure 1 Individual at rest, before (A) and after (B) injection of 25 units of Botox into the glabellar complex. The same individual attempting to frown (C), and after Botox injection (D).

D. Charting Methods

It is important for the clinician to keep organized notes in the subject's files. The immediate postinjection period is an opportune time to do this. Chart information should include the following:

1. Exact anatomical location of each injection site
2. Dosage (in units) that was delivered at each point

3. The lot number of the vial
4. The freshness of the toxin (should be within four hours)
5. The dilution used
6. The total dose administered
7. Any unusual occurrences during the procedure

VI. GLABELLAR FROWN LINES

This section illustrates the successful use of BTX-A for the treatment of wrinkles and lines in the glabellar region. The muscles in this area are associated with the frown and do not have significant clinical function, making the region an excellent candidate for our initial studies with BTX-A in cosmetic applications (3).

A. Background

The glabellar wrinkles or lines are sometimes referred to as "frown lines." When they are deep and prominent, they may give unintended social cues, such as anger, fear, or worry. The effect of these wrinkles can be so annoying that many individuals seek cosmetic treatment. Prior to the availability of BTX-A, the treatment of choice for frown lines was filling agents. Although filling materials were considered fairly safe, there were reports of allergic or idiosyncratic reactions to them (44), or complications such as

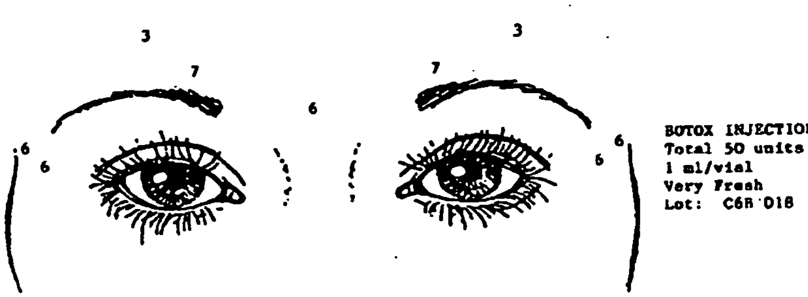

Figure 2 Diagram of BTX-A injection procedure charting for the glabellar area. The numbers indicate the number of mouse units of BTX-A deposited at each injection site. "Very Fresh" indicates that the toxin was injected within 4 hours of reconstitution.

vascular occlusion leading to in
side effects motivated the use o

B. Anatomy

The muscles controlling the fr
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The most successful cases
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C. Procedure

The technique for injecting BT
years, due to our experience in
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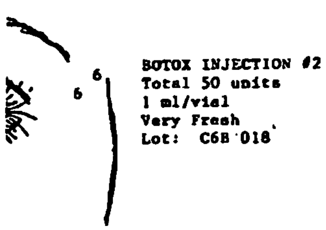

charting for the glabellar area.
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vascular occlusion leading to infarction and even blindness (45–47). These side effects motivated the use of BTX-A as an alternative therapy.

B. Anatomy

The muscles controlling the frown, although complex, do not serve any other function. This means that paralysis of the region will not result in significant untoward side effects. Muscles producing the frown include the corrugator, which moves the brow medially, the procerus and depressor supercilii, which pull the brow inferiorly, and the orbicularis, which moves the brow medially. The location, size, and use of the muscles vary markedly among individuals, so the clinician must individualize the treatment sites and dosage.

The most successful cases for BTX-A treatment of glabellar wrinkles include individuals who frown inappropriately or have type 2 Glogau wrinkles (48). The "inappropriate frowners" tend to frown subconsciously, often while concentrating or maybe during sleep. For this group, paralysis of the glabellar central brow musculature eliminates the frown, allows a more flattering expression and, in addition, relieves frontal tension. In individuals with type 2 Glogau wrinkles, however, significant weakening of the area, rather than paralysis, allows the unopposed action of the central frontalis, producing a more satisfactory "open-eyed" look.

C. Procedure

The technique for injecting BTX-A has been modified over the past few years, due to our experience in working with the toxin. The procedure presented in this chapter is different from those described by other clinicians (11–13,29) and varies from the methodology that we previously reported (9,10,49). BTX-A therapy in the glabellar regions is a safe procedure with very few side effects.

Photographs of the glabellar region should be taken when muscles are at rest and during maximal frowning to determine individual characteristics of the patients. The type of brow arch, brow asymmetry, and whether the brow is ptotic or crosses the orbital rim, can all be important factors in determining the site and dosage of the injection.

The sites of injection in the glabellar region are determined by brow shape. Generally, brows can be classified into two types: an arched, female-type brow, and a horizontal, male-type brow. The site and dosage of the injection are dependent on the brow type. For example, the male-type brow is associated with greater muscle bulk and requires more toxin to produce paresis. Another consideration is that the needle insertion

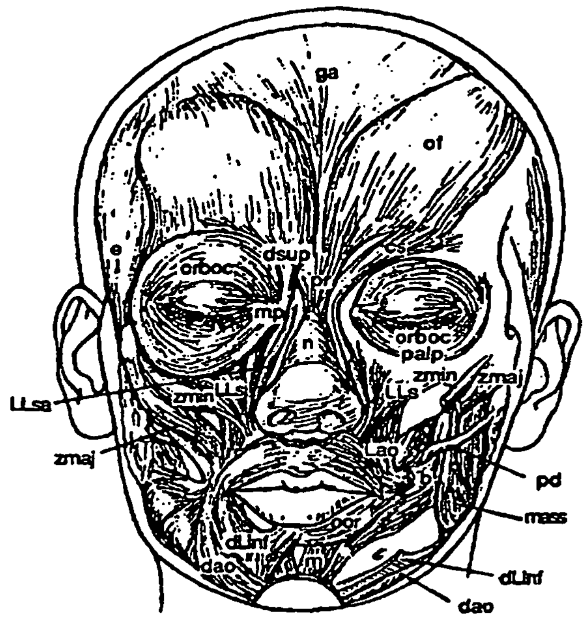

Figure 3 Anterior view of the muscles of facial expression. ga = galea aponeurotica; of = frontal belly, occipitofrontalis muscle; cs = corrugator supercilii; pr = procerus; dsup = depressor supercilii; orboc = orbicularis oculi; orboc palp = orbicularis oculi, palpebral part; e = epicranius (temporoparietalis muscle); mp = medial palpebral ligament; n = nasalis muscle; LLs = levator labii superioris; LLsa = levator labii superioris alaeque nasi; zmin = zygomaticus minor; zmaj = zygomaticus major; pd = parotid duct; dsept = depressor septi; Lao = levator anguli oris; b = buccinator; r = risorius muscle; mass = masseter; oor = orbicularis oris; dLinf = depressor labii inferioris; dao = depressor anguli oris; m = mentalis muscle. (Used by permission of Marcel Dekker, Inc., New York, NY.)

point, located near a vascular area, should be at a point where it is safe to apply postinjection pressure. The supratrochlear vessels are located immediately lateral to the site of injection, and minor bleeding commonly occurs.

Below is an overview of techniques used for injection of BTX-A into the glabellar region. At the dosages used in the treatment of the glabellar region, it is appropriate to use a tuberculin syringe with a 30-gauge needle. The dosage for each of the 7 sites is dependent on the amount of regional muscle mass and the type of brow arch. For example, in a female

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with a more male type of hori
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a greater muscle mass, the dos
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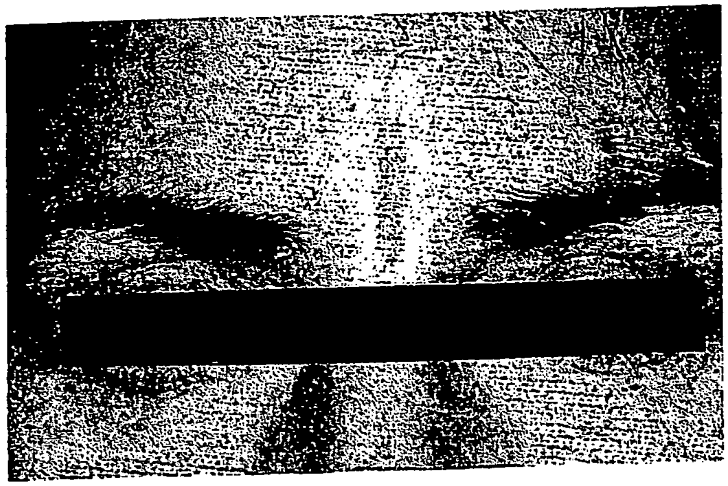

Figure 4 Individual with more horizontal brow position and deep cleft between the eyebrows due to repeated "frowning" while concentrating.

with a more male type of horizontal brow but without a greater muscle mass, 3–4 units are injected at each site. In contrast, in male or females with a greater muscle mass, the dosage should be adjusted accordingly to 5–7 units/site. This is in general agreement with doses used by other clinicians (13), although lower doses (2–4 units of Botox in each corrugator) were also reported to produce satisfactory results (29). Injections should be symmetrical to obtain a balanced look.

The dose of BTX-A (25 units are dissolved in 0.25 ml so that one unit is present in each 0.01 ml) is drawn up into the syringe and the air is expressed. The patient should be in the sitting position with the chin down and the head slightly lower than the physician's. The needle is inserted just above the eyebrow, directly above the caruncle of the inner canthus. Whatever the position of the eyebrow, the injection site should always be above the position of the bony supraorbital ridge. The thumb of the contralateral hand is used to feel the position of the supraorbital ridge by comparison with the eyebrow.

After injecting 3–5 units, *do not completely remove the needle.* Keeping it in the same injection site, slowly withdraw the needle, keeping its tip superficially beneath the skin. Reposition the needle and advance the tip superiorly so that the tip is at least 1 cm above the previous injection site in the orbicularis oculi. This is a superficial injection, as the orbicularis oculi is superficial to frontalis at this location. An additional 4–5 units of

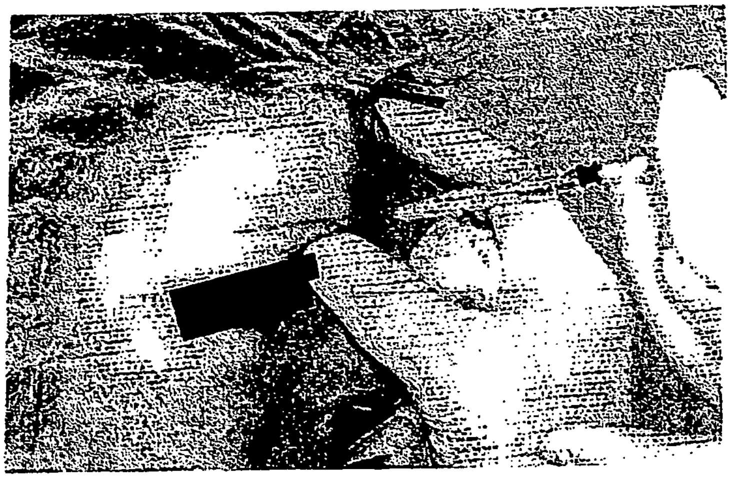

Figure 5 Injection of BTX-A behind the medial eyebrow.

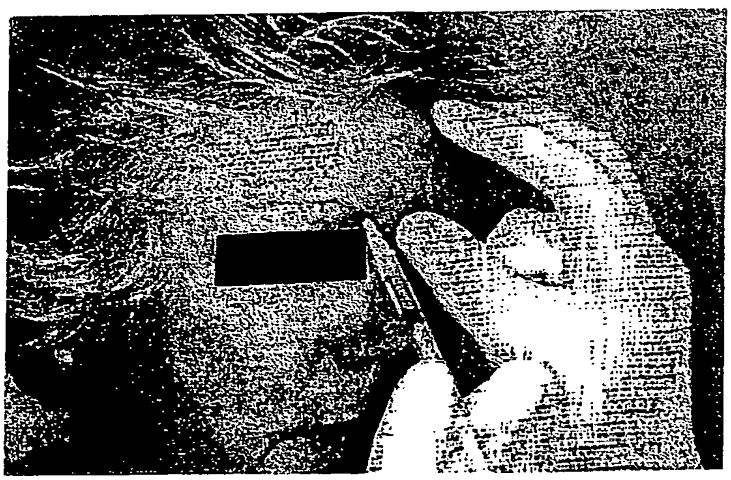

Figure 6 Injection of BTX-A above the medial eyebrow.

Figure 7 Injection into procerus b

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Another 3–5 units is inject
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During the postinjection p
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D. Follow-up

We ask that treated individuals
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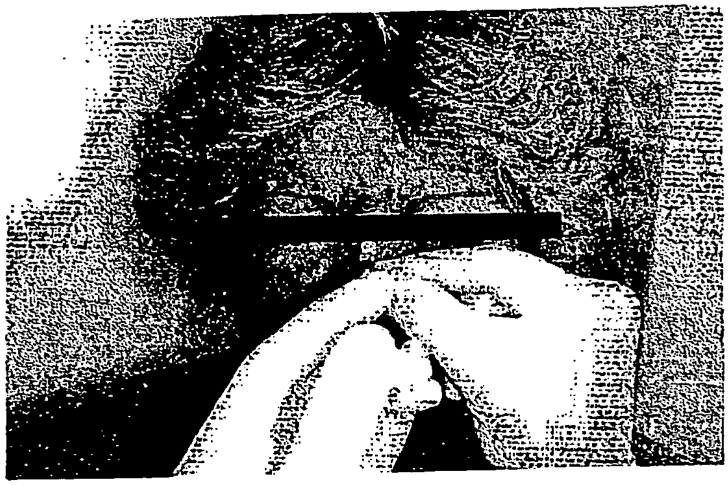

Figure 7 Injection into procerus between the eyebrows.

toxin should be injected at this site. This procedure is then repeated on the opposite side of the brow.

Another 3–5 units is injected into procerus in the midline, at a point below a line joining the brows and above the crossing point of the "X" formed by joining the medial eyebrow to the contralateral inner canthus. Finally, in many individuals, but especially in those with horizontal brows, an additional 4–5 units is injected into a point 1 cm above the supraorbital rim in the midpupillary line. It is critical to check that excessive eyebrow ptosis is not present, which is done by feeling the supraorbital rim with the thumb of the other hand.

During the postinjection period, the subject should remain vertical. Using a mirror to determine if there is any bleeding, the subject should be told to gently wipe off any blood from the treated area with a damp gauze. They should be told to frown as much as possible within the next 2–3 hours (while the toxin is binding) but not to press or manipulate the treated area.

D. Follow-up

We ask that treated individuals return in 4–6 weeks, for a follow-up examination. During the follow-up period, photographs are taken and treatment responses can be assessed. In individuals with deep glabellar frown

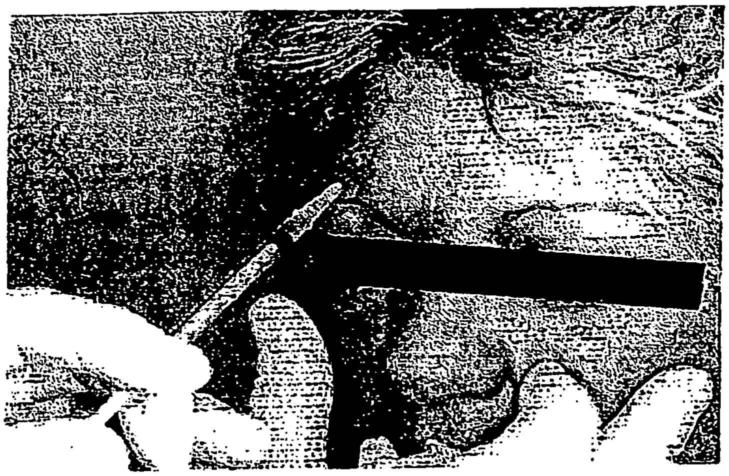

Figure 8 Injection above the center of the eyebrow.

lines, we recommend injections at 3-month intervals over a period of one year. This procedure keeps the musculature paralyzed and allows the glabellar furrows to drop out (49). After the year, they can return when they decide they need retreatment. All individuals are instructed to contact their physicians if anything unexpected occurs.

Touch-up injections are not common, and consideration should be given to the amount of time between the initial treatment and touchup. Because of the immunogenecity of the toxin, touch-up should not be given prior to 4 weeks postinjection due to the possibility of inducing antibody formation. Since the full effect of the toxin does not take place until after a period of 2 weeks, touch-up injections before 2 weeks are contraindicated.

E. Possible Complications

There are very few BTX-A–related complications in the glabellar region. At the dosages used, the toxin is very dilute and is nonparticulate; even if injection occurred intravascularly (e.g., into retinal circulation), untoward effects would be very unlikely. We have been treating patients with BTX-A for more than 13 years, with dosages ranging from 2.5 to 100 units/injection (with an average of 25 units), and none of our patients has suffered a serious, irreversible complication.

There are, however, some reversible complications associated with

BTX-A therapy. The most comm
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and the ptosis occurred midbro
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(29). The clinician should be ale
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Ptosis of the upper eyelid is
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usually lasts from 2 to 4 weeks
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The incidence of ptosis in o
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prevent ptosis include the follow

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2. The toxin should be
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3. Injected areas should
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4. The subject should re

VII. CROW'S FEET

A. Background

"Crow's feet" are wrinkles exte
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B. Anatomy

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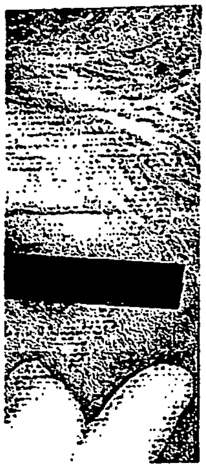

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BTX-A therapy. The most common, ptosis, usually affects the upper eyelid, although there is one case in which we injected under the frown lines and the ptosis occurred midbrow (10). Garcia & Fulton report a 5% incidence of ptosis when treating both forehead and glabellar areas together (29). The clinician should be alert to the possibility of ptosis when injecting above the lateral brow. Pretreatment assessment of this area is important before injection.

Ptosis of the upper eyelid is a result of migration of the injected toxin through the orbital septum to the upper eyelid levator muscle, producing a weak paralytic effect. It occurs 1–2 weeks *after* the cosmetic effect and usually lasts from 2 to 4 weeks. The treated individual will usually be aware of the ptosis, especially late in the day, but its effects are not cosmetically significant.

The incidence of ptosis in our clinic is low (1.7% to 3.0%) and is technique dependent (10,31). Some of our recommendations for procedures to prevent ptosis include the following:

1. The injected volume should be accurately dosed and kept to a minimum.
2. The toxin should be accurately placed and injected no closer than 1 cm above the central eyebrow.
3. Injected areas should not be manipulated for several hours postinjection.
4. The subject should remain vertical for 2 to 3 hours postinjection.

VII. CROW'S FEET

A. Background

"Crow's feet" are wrinkles extending laterally from the periorbital area and are usually a sign of aging, particularly photoaging. In most individuals, the wrinkles are produced by the contraction of muscles associated with facial expression (orbicularis oculi). By relaxing the relevant muscles, treatment of crow's feet is successful even in circumstances where the skin is severely photoaged (Glogau type 4) (48).

B. Anatomy

The muscle group involved in the production of crow's feet in the lateral periorbital area is the orbicularis oculi that ring the orbit. The lateral fibers of the orbicularis oculi are arranged in a circular pattern around the eyes. Contraction of the fibers produces forceful closure of the eyelids. Therefore, the goal of treatment of these muscles is relaxation or weakening,

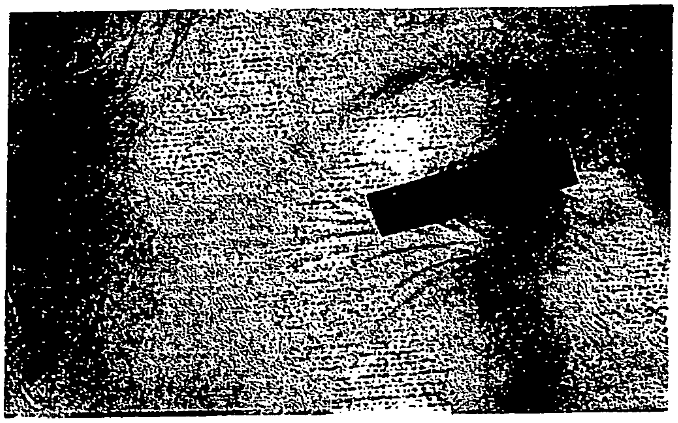

Figure 9 Individual with marked photoaged skin smiling maximally, demonstrating radiating lines from the lateral canthal area.

rather than paralysis. The lateral radiation of wrinkles from the area of the lateral canthus occurs at right angles to the muscle group, although variations in anatomy can produce different patterns.

C. Procedures

In general, 2 to 3 injection sites lateral to the lateral orbital rim are used (10,12,13,29,43,50), and equal doses of toxin are injected into each of the sites (approximately 2–5 units/site; 5–15 units/side). The dosage used in the lateral orbital region can vary. In our procedure, we start with 6 units of Botox, which are injected in equal proportions at 3 sites. The dosage can be increased to a total of 15 units (up to 5 units/site). Total dose ranges used by others include 5–15 units (mean = 7.5 units) (50) and 4–5 units of Botox (29).

We found that it is more efficient to dilute the toxin using 100 units/ml. Other clinicians use dilutions as low as 10 units/ml, since 5 units/ml were found to result in shorter responses (29). It might seem that greater dilutions would produce smoother effects, but we have not observed this clinically.

To identify the injection sites, the individual is asked to smile maximally, and the center of the crow's-feet area is noted. The first injection site is in the center of the area of maximal wrinkling, approximately 1 cm lateral to the lateral orbital rim. The second and third injections sites are approximately 1–1.5 cm above and below the first injection site, respec-

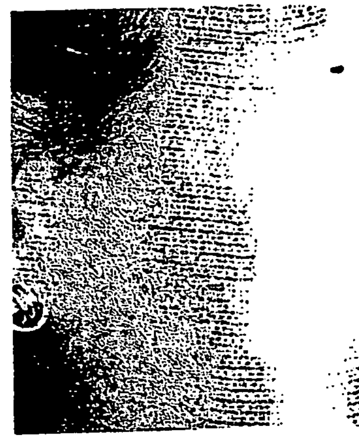

Figure 10 Injection sites for crov
inferior to the lateral canthus. 4–5

tively. In some cases, crow's fe
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D. Follow-up

In our clinic, the duration of
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receiving lower doses (6 units
to 12–15 units/side with initi
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Individuals considering
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E. Possible Complications

Ascher et al. report a worseni
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Figure 10 Injection sites for crow's feet in this individual, whose lines are mainly inferior to the lateral canthus. 4–5 units Botox is injected at each location.

tively. In some cases, crow's feet are distributed equally above and below the lateral canthus, while in others, crow's feet are primarily below the lateral canthus. In these individuals, the injection sites may be in a line that angles from anteroinferior to superoposterior. In any case, the most anterior injection should be lateral to a line drawn vertically from the lateral canthus.

D. Follow-up

In our clinic, the duration of the effect is not as long as that for treatment in glabellar regions. Since these individuals appear to have been those receiving lower doses (6 units/side), we recently have increased the dose to 12–15 units/side with initially more satisfactory results. Other groups have reported effects lasting an average of 3.6 months (43); Keen et al. recommend retreatment every 4–6 months (50).

Individuals considering treatment should be told that BTX-A injection for the treatment of crow's feet reduces but does not eradicate wrinkling in this area. Keen et al. observed an average improvement of 1.36 on a 0–3 rating scale (as assessed by the treated individuals) (50).

E. Possible Complications

Ascher et al. report a worsening of preexisting fat herniations of the lower eyelid in three cases (43). Keen et al. observed a temporary droop of the

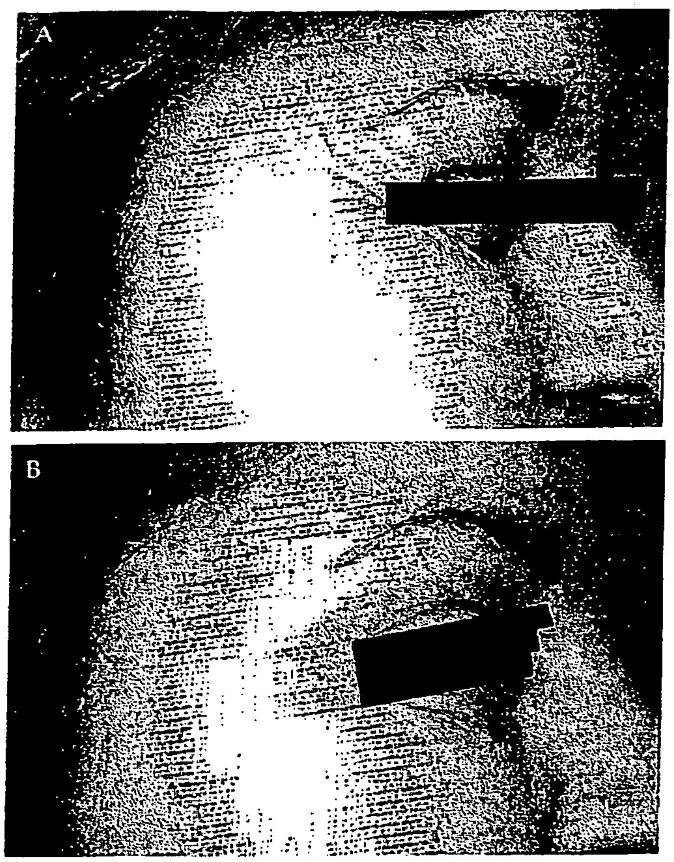

Figure 11 Individual attempting to smile maximally before (A) and after (B) injection of 12 units of Botox into lateral orbicularis oculi.

lower eyelid in approximately 5% of cases (4 of 80) (50). The eyelid droop was prevented by injecting further into the lateral canthus.

VIII. HORIZONTAL FOREHEAD LINES

A. Background

The cosmetic treatment of horizontal forehead lines with BTX-A requires a more cautious approach than does treatment of the glabellar or the lateral

periorbital region, since paraly
undesirable effects. An excessi
out weakening of the depress
depressors, producing a loweri
ated shadowing of the eyes, a
clinician should be conservat
remain intact to permit brow el
to the identification of injectio
site are recommended. This is p
ing with numerous horizontal
uals, it is necessary to weake
result in a degree of brow ptos
uals should be aware of the pos
what to expect from treatment

B. Anatomy

The forehead musculature in
underlying the forehead. It is i
posteriorly and inferiorly into
laris oculi, corrugator supercili
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gap between the two bellies of

C. Procedures

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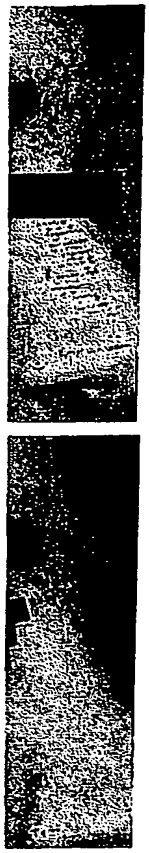
group used 40–50 units inject



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eral canthus.

lines with BTX-A requires a
›f the glabellar or the lateral

periorbital region, since paralysis in the forehead (frontalis) muscles has undesirable effects. An excessive weakening of the frontalis muscle without weakening of the depressors will result in unopposed action of the depressors, producing a lowering of the brow. Combined with an associated shadowing of the eyes, an angry appearance may result. Thus, the clinician should be conservative and allow some functional areas to remain intact to permit brow elevation. For this reason, a careful approach to the identification of injection sites and the corresponding dosages per site are recommended. This is particularly relevant to individuals presenting with numerous horizontal lines across the forehead. In these individuals, it is necessary to weaken the entire forehead muscles, which will result in a degree of brow ptosis and a loss of expressivity. These individuals should be aware of the possible outcome of this procedure and be told what to expect from treatment.

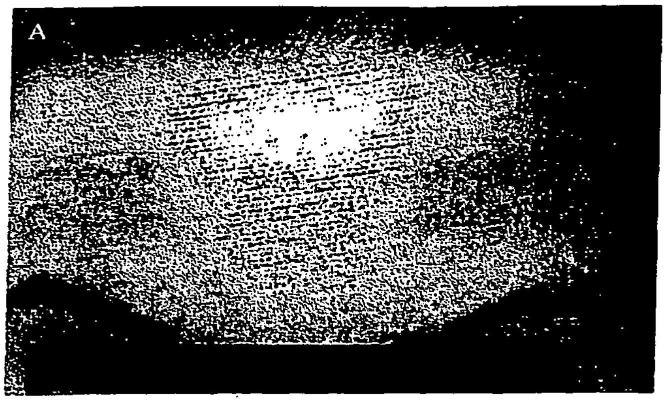
B. Anatomy

The forehead musculature includes a large, vertically oriented muscle underlying the forehead. It is inserted into the galea aponeurotica superoposteriorly and inferiorly into the skin of the brow, the procerus orbicularis oculi, corrugator supercilii, and depressor supercilii. It is the contraction of frontalis that produces horizontal forehead lines. There is a variable gap between the two bellies of frontalis medially.

C. Procedures

In treating wrinkles of the forehead, it is necessary to distinguish their extent. Softening the lines can be accomplished effectively by weakening of the frontalis. In treating individuals with 1–2 forehead lines, BTX-A (approximately 10–15 units in one-unit doses) should be injected along both sides of the line at 1–2 cm intervals. The incidence of brow ptosis is low when the injections are kept at least 1 cm above the brow.

In treatment of individuals with many forehead lines, softening of the lines can be accomplished by a careful weakening of the muscles. The clinician should be aware that injection into the lateral brow should be done carefully, as this region appears to be particularly vulnerable for the development of brow ptosis. The ability of the toxin to spread provides a mechanism by which softening of the lines can be accomplished (22). Keen et al. injected 16 sites with a total of 20 units of Botox at a dilution of 2.5 or 5 units/ml (12). Garcia and Fulton injected a total of 15–20 units of Botox at a dilution of 10 units/ml in 0.1-cc injections for glabellar wrinkles, crow's feet, and forehead lines combined (29). Using Dysport, Ascher's group used 40–50 units injected into forehead regions (43).



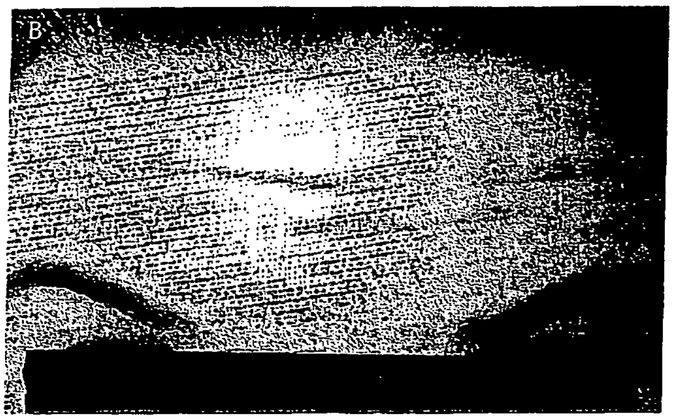


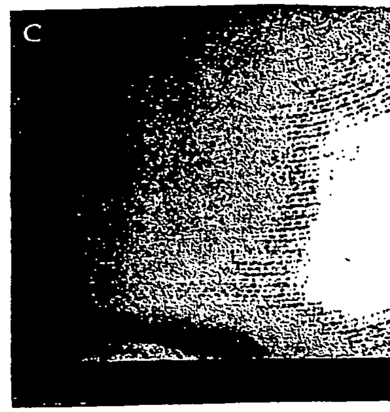
Figure 12 A and B.

D. Follow-up

The need for retreatment in the forehead region appears to be approximately 2–3 times per year, although Ascher et al. found the mean duration of treatment to be approximately 3.4 months (12,43).

E. Possible Complications

In the treatment of forehead lines, the success rates ranged from 46% to 100% (9 of 9) improvement (51,12). However, in the Keen et al. study, 2 of



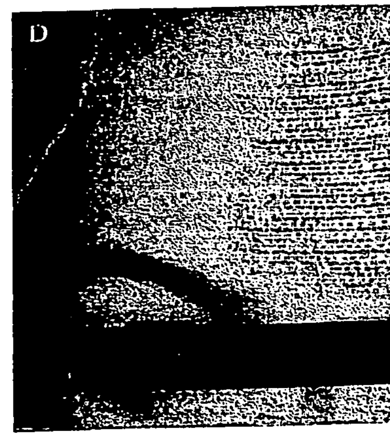


Figure 12 Individual at rest (A) a
after BTX-A injection. Note injection
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the 9 patients thought their eyeb
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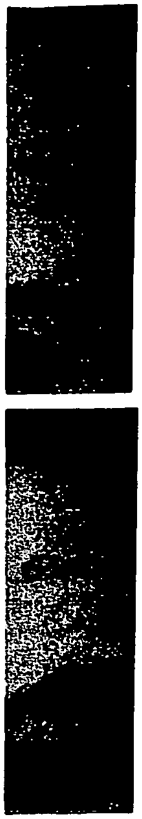
a side-by-side comparison study
others, some patients complaine
the forehead region (10,12).



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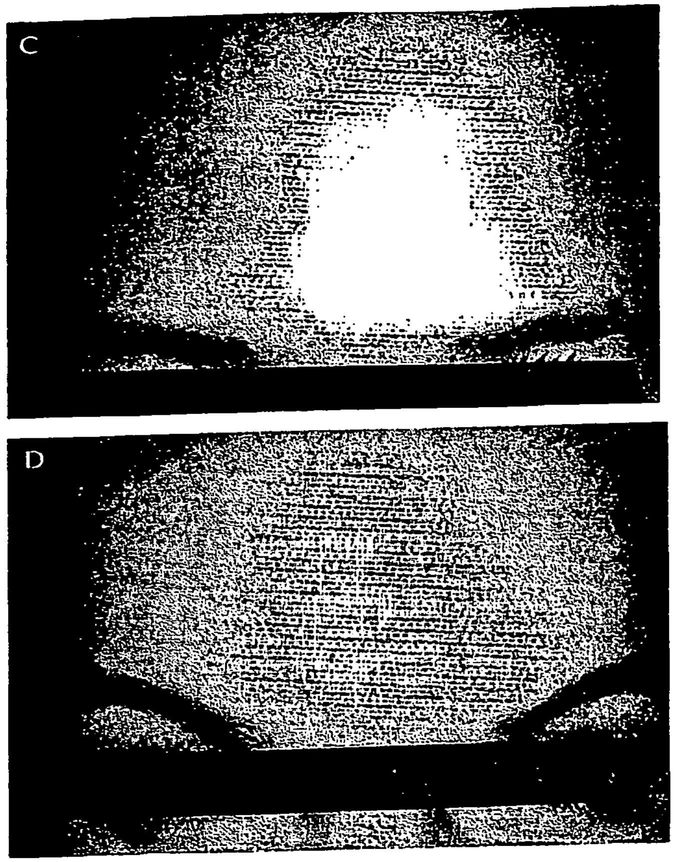

Figure 12 Individual at rest (A) and with eyebrow elevation (B) immediately after BTX-A injection. Note injection sites. 1–2 units of Botox per site. Same individual at rest (C) and attempting brow elevation (D), showing BTX-A effect.

the 9 patients thought their eyebrows had drooped slightly (12). Another one described a "heavy" forehead on the side of the toxin-injection site in a side-by-side comparison study of Botox and a control. In our studies and others, some patients complained of pain associated with the injection to the forehead region (10,12).

IX. OTHER COSMETIC USES

BTX-A has been used on other facial and neck regions with varying success. Some of these trials have been done on a small number of patients. The optimal techniques have not yet been established. In other regions (e.g., melolabial folds) and conditions (e.g., Bell's Palsy), we feel that BTX-A may help in a limited way.

A. Neck Lines

We have observed that BTX-A is a successful treatment for horizontal neck lines involving the underlying platysma muscle (10). Blitzer et al. made similar observations in a small group (N = 4 cases) in which individuals were injected with 10–20 units of Botox (mean dose = 15 units) (51). Anecdotal reports suggest that BTX-A is effective for vertical neck bands.

B. Mental Creases

Two cases in our practice have been documented (10). In the first case, the subject was injected with 10 units of Botox along the length of the crease. It appears that the dose was too large, as there was drooling and incompetence of the orbicularis oris, as well as some difficulty with speech, suggesting involvement of the mentalis and depressor labii inferioris muscles. A second subject, treated with a lower dose (5 units) of Botox, had favorable results that lasted for approximately 3 months.

C. Melolabial Folds

In the treatment of melolabial folds, Blitzer et al. reported improvement (13), but we have not been able to replicate the results. This may be because the melolabial fold is formed as a result of excess skin hanging over the skin attachment of zygomaticus major and minor, levator labii superioris and levator labii superioris alaeque nasi. Muscle relaxation in this region to smooth out the folds produces a cosmetically unacceptable ptosis of the upper lip. In addition, since the lips are very mobile, it is likely that BTX-A, in weakening the muscles, can produce results such as an asymmetrical smile, incompetent mouth, or flaccid cheek.

In our treatment, we inject 2 units of Botox into the levator labii superioris alaeque only in special cases. This results in a smoothing of the superomedial part of the melolabial fold. Treatment of the more lateral part of the fold should be done with other methods.

D. Facial Asymmetry

The most common underlying cause of facial asymmetry is Bell's Palsy. This disease leaves the affected side with residual paresis resulting in

decreased expressivity. The treatme
expressivity on the unaffected side
can result from aberrant regeneratio
requires treatment on the affected sid

X. CONTRAINDICATIONS & PREC

The list of contraindications for BTX
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BTX-A therapy is relatively
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Finally, although there are rel
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XI. CONSENT

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residual paresis resulting in

decreased expressivity. The treatment strategy with BTX-A is to reduce expressivity on the unaffected side (52–54). Alternatively, hyperkinesia can result from aberrant regeneration, or after surgical anastomosis, and requires treatment on the affected side.

X. CONTRAINDICATIONS & PRECAUTIONS

The list of contraindications for BTX-A therapy is relatively short. However, treatment with the toxin is not advised in individuals known to be hypersensitive to the ingredients in the commercial formulation of BTX-A (for Botox, this includes human albumin and saline); individuals with muscular or neurological diseases; or (3) pregnant women.

Individuals preparing for therapy with BTX-A should give a routine medical history. Particular attention should be given to possible neurological diseases, especially those involving the NMJ. Although some neurological patients have been treated successfully (55), the risk is that BTX-A may unmask clinical disease (56); therefore, neurological consultation is recommended for individuals experiencing symptoms of neurological and/or muscular disease.

BTX-A therapy is relatively contraindicated for patients taking aminoglycosides. This class of drugs interferes with neuromuscular transmission and has been reported to potentiate the effects of BTX-A (18,57–59). However, at the therapeutic dosages used in cosmetic therapy, there are no reports of BTX-A and aminoglycoside interactions.

Finally, although there are relatively few reports on teratogenicity, the BTX-A toxin is not approved for use in pregnant women, and we do not use it in lactating women. To our knowledge, only one study was completed on BTX-A and pregnancy (60). In this study of 9 pregnant women administered BTX-A (dosage not reported), one delivered prematurely. The premature delivery was not thought to be related to the toxin.

XI. CONSENT

The consent form serves many different roles, and it is important for the clinician to obtain a properly signed consent form prior to treatment. The consent form for the treatment of facial wrinkles with Botox in our clinic includes sections on rationale, results and postoperative care, and risks and complications, the consent to take photographs, exclusion criteria and finally, payment. The form should be reviewed carefully with the individual seeking treatment, with mention given to the mechanism of action, the expected results, and the timing of BTX-A effects. In particular, the indi-

vidual should be aware of the small and unlikely chance (less than 3%) of developing eyelid ptosis. In addition, patients should be advised that other temporary side effects—including occasional numbness in a small area of the forehead, bruising, and transient headache—may also occur. Prior to treatment, emphasis should be on the reversibility of the "drooping eyelid," which lasts only 2–4 weeks. Headaches and other side effects last for approximately 2–3 hours.

Patients should be warned of the unlikely occurrence of being treatment-resistant to BTX-A therapy. They should also be informed that the duration of effect may be shorter or longer than expected.

XII. PHYSICIAN TRAINING

Training guidelines for the use of botulinum toxin for the treatment of neurologic disorders has been published by the Therapeutic and Technology Assessment Subcommittee of the American Academy of Neurology (61). We recommend the use of these guidelines for learning about the use of BTX-A for cosmetic applications, as many of the techniques are the same. We also recommend attending a course on the cosmetic use of BTX-A, including live patient demonstrations, prior to performing the technique. The authors offer a one-day course (including a live demonstration) for Botox therapy in cosmetic applications.

XIII. SUMMARY AND CONCLUSIONS

We feel that treatment with BTX-A is very safe and simple for the clinician to administer. Since 1988, when we first used BTX-A for cosmetic applications (3), we have experienced a high rate of success in treating facial lines and wrinkles. We also find that the duration of effects of the toxin may increase over time with repeated treatments. A few cases have therapeutic effects lasting for one year or longer. BTX-A now has been demonstrated to have a role to play in the management of some cosmetic problems.

REFERENCES

1. Middlebrook JL. Cell surface receptors for protein toxins. In: Simpson LL, ed. Botulinum neurotoxin and tetanus toxin. San Diego: Academic Press, 1989; 7:153–178.

2. Jankovic J, Brin MF. Therapeu 1991;324(17):1186–1194.
3. Carruthers A, Carruthers J. Annual meeting of the Americ Florida, Mar 13–17, 1991.
4. Scott AB. Botulinum toxin inje to strabismus surgery. Ophthal
5. Scott AB, Rosenbaum A, Colli lar muscles. Invest Ophthalmo
6. Scott AB. Botulinum toxin in Trans Am Ophthalmol Soc 198
7. Scott AB, Kennedy EG, Stubbs for blepharospasm. Arch Opht
8. Brin MF. Botulinum toxin: Ch clinical use of botulinum toxi York, Oct 1991:19.
9. Carruthers JDA, Carruthers J botulinum-A exotoxin. J Derm
10. Carruthers A, Carruthers JD glabellar frown lines and othe Therapy with botulinum toxi
11. Guyuron B, Huddleston SW. tion. Plast Reconstr Surg 1994;
12. Keen M, Blitzer A, Aviv J, et al. Results of a double-blind, plac 94;94–99.
13. Blitzer A, Brin MF, Keen MF, e functional lines of the face. Ar 1022.
14. Simpson LL. Peripheral actio Botulinum neurotoxin and te 1989:153–178.
15. Simpson LL. The structure (Abstract). Mov Disord 1995;
16. Black JD, Dolly JO. Interacti nerve terminals: 1 Ultrastruc tation of distinct membrane r Biol 1986;103:521–534.
17. Black JD, Dolly JO. Interacti nerve terminals: II. Autorad nerves by acceptor-mediated
18. Yamada S, Kuno Y, Iwanaga neuromuscular junction: Par 130–138.
19. Coffield JA, Considine RV, Si

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rotein toxins. In: Simpson LL, ed.
an Diego: Academic Press, 1989;

2. Jankovic J, Brin MF. Therapeutic uses of botulinum toxin. New Eng J Med 1991;324(17):1186–1194.
3. Carruthers A, Carruthers J. Botulinum toxin use for glabellar wrinkles. Annual meeting of the American Society for Dermatologic Surgery, Orlando, Florida, Mar 13–17, 1991.
4. Scott AB. Botulinum toxin injection into extraocular muscles as an alternative to strabismus surgery. Ophthalmol 1980;87:1044–1049.
5. Scott AB, Rosenbaum A, Collins CC. Pharmacologic weakening of extraocular muscles. Invest Ophthalmol 1973;12:924–927.
6. Scott AB. Botulinum toxin injection of eye muscles to correct strabismus. Trans Am Ophthalmol Soc 1981;79:734–770.
7. Scott AB, Kennedy EG, Stubbs HA. Botulinum A toxin injection as a treatment for blepharospasm. Arch Ophthalmol 1985;103:347–350.
8. Brin MF. Botulinum toxin: Chemistry, pharmacology and immunology: The clinical use of botulinum toxin. College of Physicians and Surgeons of New York, Oct 1991:19.
9. Carruthers JDA, Carruthers JA. Treatment of glabellar frown lines with C. botulinum-A exotoxin. J Dermatol Surg Oncol 1992;18:17–21.
10. Carruthers A, Carruthers JDA. Botulinum toxin used in the treatment of glabellar frown lines and other facial wrinkles. In Jankovic J, Hallett M, eds. Therapy with botulinum toxin. New York: Marcel Dekker, 1994:577–595.
11. Guyuron B, Huddleston SW. Aesthetic indications for botulinum toxin injection. Plast Reconstr Surg 1994;93:913–918.
12. Keen M, Blitzer A, Aviv J, et al. Botulinum toxin A for hyperkinetic facial lines: Results of a double-blind, placebo-controlled study. Plast Reconstr Surg 1994a; 94;94–99.
13. Blitzer A, Brin MF, Keen MF, et al. Botulinum toxin for the treatment of hyperfunctional lines of the face. Arch Otolaryngol Head Neck Surg 1993;119:1018–1022.
14. Simpson LL. Peripheral actions of the botulinum toxins. In: Simpson LL, ed. Botulinum neurotoxin and tetanus toxin. San Diego, CA: Academic Press, 1989:153–178.
15. Simpson LL. The structure and mechanism of action of botulinum toxin (Abstract). Mov Disord 1995;10:362.
16. Black JD, Dolly JO. Interaction of ^{125}I-labelled botulinum neurotoxins with nerve terminals: 1 Ultrastructural autoradiographic localization and quantitation of distinct membrane receptors for type A and B on motor nerves. J Cell Biol 1986;103:521–534.
17. Black JD, Dolly JO. Interaction of ^{125}I-labelled botulinum neurotoxins with nerve terminals: II. Autoradiographic evidence for its uptake into motor nerves by acceptor-mediated endocytosis. J Cell Biol 1986;103:535–544.
18. Yamada S, Kuno Y, Iwanaga H. Effects of aminoglycoside antibiotics on the neuromuscular junction: Part I. Int J Clin Pharmacol Ther Toxicol 1986;24: 130–138.
19. Coffield JA, Considine RV, Simpson LL. The site and mechanism of action of

botulinum neurotoxin. In: Jankovic J, Hallett M, eds. Therapy with botulinum toxin. New York: Marcel Dekker, 1994:3–13.
20. de Paiva A, Ashton AC, Foran P, et al. Botulinum A like type B and tetanus toxins fulfil criteria for being a zinc-dependent protease. J Neurochem 1993; 61:238–2341.
21. Schiavo G, Rosetto O, Benfenati F, et al. Tetanus and botulinum neurotoxins are zinc proteases specific for components of the neuroexocytosis apparatus. Ann NY Acad Sci 1994;710:65–75.
22. Borodic GE, Ferrante RJ, Pearce LB, et al. Pharmacology and histology of the therapeutic application of botulinum toxin. In: Jankovic J, Hallett M. eds. Therapy with botulinum toxin. New York: Marcel Dekker, 1994:119–157.
23. Duchen LW. Changes in motor innervation and cholinesterase localization induced by botulinum toxin in skeletal muscle of mouse: Differences between fast and slow muscles. J Neurol Neurosurg Psychiatry 1970;33:40–54.
24. Hassan SM, Jennekens FG, Wieneke, et al. Elimination of superfluous neuromuscular junctions in rat calf muscles recovering from botulinum toxin-induced paralysis. Muscle Nerve 1994; 17:623–631.
25. Holds JB, Alderson K, Fogg SG, et al. Terminal nerve and motor end plate changes in human orbicularis muscle following botulinum A exotoxin injection. Invest Ophthalmol Vis Sci 1990;31:178–181.
26. Scott AB, Suzuki D. Systemic toxicity of botulinum toxin by intramuscular injection in the monkey. Mov Disor 1988;3:333–335.
27. Meyer KF, Eddie B. Perspectives concerning botulism. Z Hygiene Infection Krankheiten 1951;133:255–263.
28. Lamanna C, Hillowalla RA, Alling CC. Buccal exposure to botulinal toxin. J Infect Dis 1967;117:327–331.
29. Garcia A, Fulton JE. Cosmetic denervation of the muscles of facial expression with botulinum toxin. Dermatol Surg 1996;22:39–43.
30. Gartland MG, Hoffman HT. Crystalline preparation of botulinum toxin type A (BOTOX): Degradation impotency with storage. Otolaryngol Head Neck Surg 1993;108:135–140.
31. Carruthers A, Carruthers J. Cosmetic uses of botulinum toxin. In: Coleman WP, Hanke, W, Alt T Asken S, eds. Cosmetic surgery of the skin, Second edition. New York, 1997:231–235.
32. Tsui JK, Wong NLM, Wong E, et al. Production of circulating antibodies to botulinum-A toxin in patients receiving repeated injections for dystonia (Abstract). Ann Neurol 1988;23:181.
33. Dezfulian M, Bitar R, Bartlett J. Kinetics study of immunological response to clostridium botulinum toxin. J Clin Microbiol 1987;25:1336–1337.
34. Clarke CE. Therapeutic potential of botulinum toxin in neurologic disorders. Quart J Med 1992;299:197–205.
35. Jankovic J, Schwartz K. Clinical correlates of response to botulinum toxin injections. Arch Neurol 1991;48:1253–1256.
36. Bertrand C, Molina-Negro P, Bouvier G, et al. Observations and results in 131 cases of spasmodic torticollis after selective denervation. Appl Neurophsiol 1987;50:319–323.

37. Hambleton P, Cohen HE, P
treatment. Br Med J 1992;304
38. Zuber M, Sebald M, Bathier
tients treated with type A b
rology 1993;43:1715–1718.
39. Moyer E, Setler PE. Botulin
rience. In: Jankovic J, Halle
York: Marcel Dekker, 1994:7
40. Rhew K, Ludlow CL, Karp
F. In: Jankovic J, Hallett M,
Marcel Dekker 1994:323–32
41. Scott AB, Magoon EH, McN
in children. Trans Am Opht
42. Lowe NJ, Maxwell A, Harp
double-blind, placebo cont
technique. J Am Acad Derm
43. Ascher B, Klap P, Marion
treatment des rides fronto-
Plast Esthet 1995;40:67–76.
44. Stegman SJ, Chu S, Arms
implant: Clinical and hist
13(suppl 1):39.
45. Cucin RL, Barek O. Com
Reconstr Surg 1983;71:731.
46. Driezen NG, Framm L. Su
injection in the glabellar are
47. Teimourian B. Blindness f
92:361.
48. Glogau R. Chemical peelin
49. Carruthers A, Kiene K, Carr
matology. J Am Acad Derm
50. Keen M, Kopelman JE, Aviv
periorbital wrinkles. Facial
51. Blitzer A, Binder WJ, Aviv J
with botulinum toxin: A
patients. Arch otolaryngol
52. May M, Croxon GR, Klein
EMG rehabilitation, botuli
229.
53. Clark RP Berris CE. Botulin
by facial nerve paralysis. P
54. Smet-Dieleman H, van de
injection in patients with fa
1993;47:2359–2363.
55. Brin MF. Interventional ne
local injection of botulinum

t M, eds. Therapy with botulinum

ılinum A like type B and tetanus
lent protease. J Neurochem 1993;

anus and botulinum neurotoxins
of the neuroexocytosis apparatus.

ıarmacology and histology of the
ı. In: Jankovic J, Hallett M. eds.
Marcel Dekker, 1994:119–157.
n and cholinesterase localization
cle of mouse: Differences between
Psychiatry 1970;33:40–54.
limination of superfluous neuro-
covering from botulinum toxin-
23–631.
ıinal nerve and motor end plate
ving botulinum A exotoxin injec-
-181.
otulinum toxin by intramuscular
33–335.
g botulism. Z Hygiene Infection

cal exposure to botulinal toxin. J

f the muscles of facial expression
2:39–43.
paration of botulinum toxin type
storage. Otolaryngol Head Neck

of botulinum toxin. In: Coleman
: surgery of the skin, Second edi-

ction of circulating antibodies to
epeated injections for dystonia

dy of immunological response to
ol 1987;25:1336–1337.
ım toxin in neurologic disorders.

of response to botulinum toxin

. Observations and results in 131
denervation. Appl Neurophsiol

37. Hambleton P, Cohen HE, Palmer BJ, et al. Antitoxins and botulinum toxin treatment. Br Med J 1992;304:959–960.
38. Zuber M, Sebald M, Bathien N, et al. Botulinum antibodies in dystonic patients treated with type A botulinum toxin: frequency and significant. Neurology 1993;43:1715–1718.
39. Moyer E, Setler PE. Botulinum toxin type B: Experimental and clinical experience. In: Jankovic J, Hallett M, eds. Therapy with botulinum toxin. New York: Marcel Dekker, 1994:71–85.
40. Rhew K, Ludlow CL, Karp B, et al. Clinical experience with botulinum toxin F. In: Jankovic J, Hallett M, eds. Therapy with botulinum toxin. New York: Marcel Dekker 1994:323–328.
41. Scott AB, Magoon EH, McNeer KW, et al. Botulinum treatment of strabismus in children. Trans Am Ophthalmol Soc 1990;87:174–180.
42. Lowe NJ, Maxwell A, Harper H. Botulinum A exotoxin for glabellar folds: A double-blind, placebo controlled study with an electromyographic injection technique. J Am Acad Dermatol 1996;35:569–572.
43. Ascher B, Klap P, Marion MH, Chanteloub F. La toxine botulique dans le treatment des rides fronto-glabellaires et de al region orbitair. Annales Chir Plast Esthet 1995;40:67–76.
44. Stegman SJ, Chu S, Armstrong R. Adverse reactions to bovine collagen implant: Clinical and histologic features. J Dermatol Surg Oncol 1988; 13(suppl 1):39.
45. Cucin RL, Barek O. Complications of injectable collagen implant. Plast Reconstr Surg 1983;71:731.
46. Driezen NG, Framm L. Sudden unilateral visual loss after autogenous fat injection in the glabellar area. Am J Ophthalmol 1989;107:361.
47. Teimourian B. Blindness following fat injection. Plast Reconstr Surg 1988; 92:361.
48. Glogau R. Chemical peeling in aging skin. J Geriatr Dermatol 1994;2:30–35.
49. Carruthers A, Kiene K, Carruthers J. Botulinum A exotoxin use in clinical dermatology. J Am Acad Dermatol 1996;34:788–797.
50. Keen M, Kopelman JE, Aviv et al. Botulinum toxin: A novel method to remove periorbital wrinkles. Facial Plast Surg 1994b;10:141–146.
51. Blitzer A, Binder WJ, Aviv JE. The management of hyperfunctional facial lines with botulinum toxin: A collaborative study of 210 injection sites in 162 patients. Arch otolaryngol Head and Neck Surg (in press).
52. May M, Croxon GR, Klein SR. Bell's palsy, management and sequelae using EMG rehabilitation, botulinum toxin and surgery. Am J Otology 1989:220–229.
53. Clark RP Berris CE. Botulinum toxin: a treatment for facial asymmetry caused by facial nerve paralysis. Plast Reconstr Surg 1989;84:535–355.
54. Smet-Dieleman H, van de Heyrung PH, tassigrin MJ. Botulinum toxin A injection in patients with facia nerve palsy. Acta Rhino Laryngologica Belgica 1993;47:2359–2363.
55. Brin MF. Interventional neurology: Treatment of neurological conditions with local injection of botulinum toxin. Arch de Neurobiol 1991a;3:7–23.

56. Ergbuth F, Claus D, Engelhardt A et al. Systemic effect of botulinum toxin injections unmasks subclinical Lambert-Eaton myasthenic syndrome. Letter. J Neurol Neurosurg Psychiatry 1993;56:1235–1236.
57. Sande MA, Mandell GL. Antimicrobial agents: the aminoglycosides. In: Gilman AG, Goodman LS, Gilman A, eds. The pharmacologic basis of therapeutics. 6th ed. New York: MacMillan, 1980:1162–1180.
58. Santos JI, Swensen P, Glagow LA. Potentiation of clostridium botulinum toxic amnioglycoside antibiotics: Clinical and laboratory observations. Pediatrics 1981;68:50–54.
59. Wang YC, Burr DH, Korthals GC, et al. Acute toxicity of aminoglycoside antibiotics as an aid in detecting botulism. Appl Environ Microbiol 1984;48:951–955.
60. Scott AB. Clostridial toxins as therapeutic agents. In: Simpson LL, ed. Botulinum neurotoxin and tetanus toxin. New York: Academic Press, 1989:399–412.
61. Therapeutic and Technology Assessment Subcommittee of the American Academy of Neurology. Training guidelines for the use of botulinum toxin for the treatment of neurologic disorders. 1994;2401–2403.

12
Injectable Liquid S
New Perspectives

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I. INTRODUCTION

A Nottingham chemist, F. S. Kip
the early 1900s, when he synthes
icone-containing compounds nc
much impressed with silicones,
tifically interesting but had no
for the synthesis of silicones fol
rick, and McGregor during the
lead to the production of Dow
greasy material used to waterp
flight at higher altitudes (1).

Silicones, versatile and ea
biologically nontoxic, chemical
by exposure to a wide range
pounds, whose solidity is a fun
can exist as solids (elastomers),

Technologically advanced
silicones by the ton. Antiflatule
also present in paint thinners,
joint prosthesis, contact lenses
penile, testicular, breast, and

noted. In our experience, few patients with life-threatening hemangiomas can be considered excellent responders at current dosages.[2] Unfortunately, there is no available predictive factor of response. In our series of 7 patients, only 2 infants with facial hemangiomas had a dramatic improvement after 1 month of treatment, 1 case was stabilized, and 4 cases did not respond after 2 months of treatment or had adverse effects (hepatitis).

Second, Tamayo et al conclude that "interferon alfa-2b therapy has proved to be a good option for the treatment of infants with steroid-resistant . . . giant hemangiomas." However, in their article, only 1 infant had a corticosteroid-resistant hemangioma (case 1); for the other patients, corticosteroid therapy is not mentioned or the dosage is too low to produce resistance (1 mg/kg in case 7). We agree with Frieden[3] that systemic corticosteroids should remain the first-line treatment despite their adverse effects because most of these secondary effects are transitory and well characterized. Concerning interferon therapy, major neurologic sequelae have been described,[4] and long-term effects of treatment are not known.

Since no alternative antiangiogenic drugs are available,[5] treatment with interferon should be considered an option in the management of life-threatening hemangiomas, but only in the case of corticosteroid resistance. Because of the self-involution of hemangiomas, the efficacy of a treatment must be interpreted prudently.

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1. Tamayo L, Ortiz DM, Orozco-Covarrubias L, et al. Therapeutic efficacy of interferon alfa-2b in infants with life-threatening giant hemangiomas. *Arch Dermatol*. 1997;133:1567-1571.
2. Léauté-Labrèze C, Labbé L, Taïeb A, et al. Alarming hemangiomas: 7 cases treated with interferon alpha-2b. *Ann Dermatol Venereol*. 1998;125:174-178.
3. Frieden IJ. Which hemangiomas to treat—and how? *Arch Dermatol*. 1997;133:1593-1595.
4. Barlow C, Priebe CJ, Mulliken JB, et al. Spastic diplegia as a complication of interferon alfa-2a treatment of hemangiomas of infancy. *J Pediatr*. 1998;132:527-530.
5. Eeckhout I, Léauté-Labrèze C, Taïeb A. Pourquoi les hemangiomes immatures régressent-ils? *Ann Dermatol Venereol*. 1997;124:800-804.

In reply

We agree with Léauté-Labrèze and Taïeb in that not all of our reported cases responded dramatically to interferon therapy.

Although the literature indicates 7 years for complete spontaneous involution of hemangiomas,[1-4] we are aware of the great variability that exists among individual cases. Taking as a reference our series of more than 1000 patients with hemangioma, we considered that even the slow responders responded to treatment with interferon much faster than would have been expected by spontaneous involution only.

Our conclusion on the use of interferon alfa-2b for the treatment of corticosteroid-resistant giant or life-threatening hemangiomas was based not only on our experience but on the experience of other authors quoted in our article (references 11, 14, and 16).

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1. Solomon LM, Esterly NB. *Neonatal Dermatology*. Philadelphia, Pa: WB Saunders Co; 1973:71.
2. Hurwitz S. *Clinical Pediatric Dermatology*. Philadelphia, Pa: WB Saunders Co; 1981:192.
3. Ruiz-Maldonado R. Angiomas. In: Ruiz-Maldonado R, Parish LC, Beare JM, eds. *Textbook of Pediatric Dermatology*. New York, NY: Grune & Stratton Inc; 1989:745.
4. Wagner AM, Hansen RC. Neonatal skin and skin disorders. In: Schachner Hansen RC, ed. *Pediatric Dermatology*. New York, NY: Churchill Livingstone Inc; 1995:279.

Follow-up of Patients With Axillary Hyperhidrosis After Botulinum Toxin Injection

We read with interest the article by Naumann et al[1] on botulinum toxin therapy for focal hyperhidrosis and would like to contribute our own findings regarding the long-term efficacy of botulinum toxin therapy, as well as the putative effects on apoeccrine secretion. After our initial reports on the efficacy of high-dose (500 U per axilla) botulinum toxin A (Dysport, Ipsen Biotech, Paris, France) in a series of selected patients with severe axillary[2] and compensatory[3] hyperhidrosis (sweat secretion up to 800 mg/min), we recently conducted a 1-year follow-up (M.H., S. Breit, MD, A. C-B., and G.P., unpublished data, May 1998). Nine of 12 patients were still satisfied with sweat control that was consistently below 100 mg/min measured using gravimetry on several occasions. Three patients showed mitigated recurrence of axillary hyperhidrosis after 3, 4, and 7 months, respectively, which could be overcome by a second injection of botulinum toxin A with lasting efficacy. It should be noted that at present 2 preparations of botulinum toxin A are commercially available (Botox, Allergan, Irvine, Calif, and Dysport, Ipsen Biotech), which have to be distinguished regarding their respective dosage.

Naumann et al[1] reported a notable persistence of odoriferous sweat production in patients with axillary hyperhidrosis treated with botulinum toxin A that they ascribed to the innervating fibers of apocrine glands, which are generally adrenergic while those of eccrine glands are cholinergic and therefore are blocked selectively by botulinum toxin A. However, up to 45% of all sweat glands in the axillary region belong to an otherwise unique type, the so-called apoeccrine sweat gland.[4] Functional studies revealed higher cholinergic sensitivity and a 7-fold increased secretion rate in apoeccrine glands compared with eccrine glands.[5] Thus, botulinum toxin A may putatively affect apocrine secretion by blocking apoeccrine glands in the axillae.

In our own experience, odoriferous sweat production has not been the leading concern of patients with axillary hyperhidrosis, yet it was rated as "rather reduced" on specific questioning after patients received an injection of botulinum toxin A. Since neither the findings of Naumann et al[1] nor our own observations have been measured, attention should be given to this issue in further studies.

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1. Naumann M, Hofmann U, Bergmann I, Hamm H, Toyka KV, Reiners K. Focal hyperhidrosis: effective treatment with intracutaneous botulinum toxin. *Arch Dermatol.* 1998;134:301-304.
2. Heckmann M, Breit S, Ceballos-Baumann A, Schaller M. Axillary hyperhidrosis: successful treatment with botulinum-toxin-A. *Hautarzt.* 1998;49:101-103.
3. Heckmann M, Ceballos-Baumann A, Schaller M, Plewig G. Botulinum beyond wrinkles. *Dermatol Surg.* 1997;23:1221-1222.
4. Sato K, Lidal R, Sato F. Morphology and development of an apoeccrine sweat gland in human axillae. *Am J Physiol.* 1987;252:181-187.
5. Sato K, Sato F. Sweat secretion by human axillary apoeccrine sweat gland in vitro. *Am J Physiol.* 1987;252:166-180.

VIGNETTES

Evaluation of Psoralen–UV-B (311-nm) Bath Phototoxicity

Comparison of conventional psoralen–UV-A (PUVA) bath therapy with 311 nm of UV-B light after a bath with 0.15% solution of methoxsalen revealed the therapeutic efficacy of the narrow-band UV-B (311-nm) light in combination with methoxsalen bath (psoralen–UV-B [PUVB]) therapy in patients with psoriasis.[1,2] However, no standardized guidelines exist for PUVB bath therapy. Therefore, the aim of our study was to determine the optimal time between methoxsalen bath treatment and UV-B irradiation, as well as the persistence of photosensitivity in healthy skin after PUVB bath treatment.

A UV-B (311-nm) dose causing the first faint but sharply bordered erythema was classified as the minimal erythema dose (MED), and an analogous dose in combination with methoxsalen bath therapy was classified as the minimal phototoxic dose (MPD).

Subjects, Materials, and Methods. Ten healthy volunteers (5 women and 5 men; age range, 26-48 years; Fitzpatrick skin types II-III) were enrolled in this study after giving their informed consent. The volar site of one forearm was exclusively irradiated with doses of UV-B light ranging from 0.25 to 1.25 J/cm² (311 nm, Philips TubeLight TL 01, Philips, Hamburg, Germany) to determine the UV-B erythematogenicity. Furthermore, the opposite forearm was immersed for 20 minutes in a 5-mg/L water solution of 0.15% methoxsalen. Immediately and 1 hour after the bath, doses ranging from 0.25 to 1.0 J/cm² of UV-B (311-nm) light were applied to the opposite forearm to assess the additional phototoxic effect of methoxsalen.

For the determination of MED or MPD, the erythema readings were performed at 24, 48, 72, and 96 hours after irradiation.

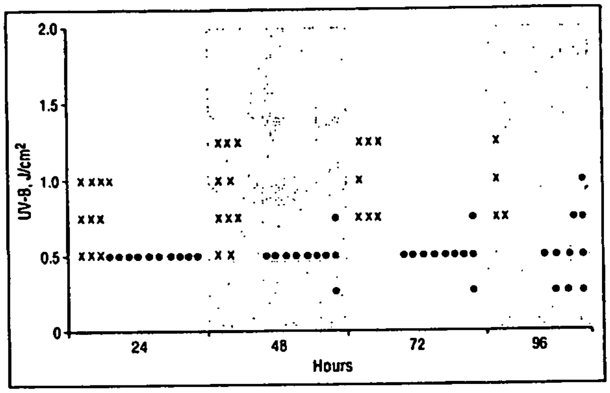

Figure 1. *Minimal erythema dose at 24, 48, 72, and 96 hours after UV-B (311-nm) irradiation treatment (Xs) and minimal phototoxic dose immediately after methoxsalen bath therapy (circles).*

Results. The lowest MED values of skin areas irradiated only with UV-B light were observed 24 hours after irradiation, followed with a statistically significant ($P<.001$) increase up to 96 hours (**Figure 1**). Since we were interested in the interaction of UV-B (311-nm) light and methoxsalen therapy, we analyzed the MED (24-hour) values from skin sites irradiated only with 311 nm of UV-B light and the MPD (24-hour) values of areas irradiated immediately as well as 1 hour after the methoxsalen bath.

In sites irradiated only with 311 nm of UV-B light, the MED (24-hour) values ranged from 0.5 to 1.0 J/cm². The same range of MPD (24-hour) values (0.5-1.0 J/cm²) were observed in areas irradiated with 311 nm of UV-B light 1 hour after methoxsalen bath therapy (**Figure 2**). The statistical analyses revealed no significant differences between these ranges ($P<.62$).

Compared with 311 nm of UV-B irradiation alone, the combination of methoxsalen bath therapy and 311 nm of UV-B irradiation immediately after the bath led to a significantly lower ($P<.01$) MPD value of 0.5 J/cm² (Figure 2).

Comment. Whereas in PUVA treatment the phototoxic effects can be put on par with the phototoxic properties of psoralen owing to the almost complete lack of erythematogenicity of UV-A light at routinely used doses, UV-B irradiation itself is erythematogenic. Thus, only a decrease of the UV-B–induced MED values can be ascribed to phototoxic properties of methoxsalen.

Our results demonstrate that the MPD of UV-B (311-nm) light was significantly decreased if the skin was irradiated with UV-B (311 nm) light immediately after bath therapy because of the additional phototoxic effect of methoxsalen.

However, UV-B (311-nm) irradiation treatment 1 hour after the bath revealed MPD values similar to MED

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ORIGINAL COMMUNICATION

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Long-term treatment of cervical dystonia with botulinum toxin A: efficacy, safety, and antibody frequency

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Abstract Data from 616 patients suffering from idiopathic cervical dystonia were analyzed to determine the efficacy and safety of treatment with botulinum neurotoxin type A (BoNT/A). Since the specific purpose of this study was to determine the long-term effects of this treatment, the analysis focused specifically on the patients ($n = 303$) having received six or more injections. Statistical analysis of a standardized documentation showed sustained significant benefit as measured by a disease severity score independent of the type of cervical dystonia. Furthermore, pronounced individual differences were found in response to this treatment although initial clinical scores and doses of BoNT/A were similar. There was no indication of previously unknown adverse events, the only risk being the development of serum antibodies against the toxin. As in previous studies on short-term effects of BoNT/A treatment, the most frequent adverse event was dysphagia, which occurred on average 9.7 days after injection and lasted on average 3.5 weeks. While secondary nonresponse was seen in approx. 5% of patients, antibody tests revealed neutralizing serum antibodies in only 2%. On the basis of the present data, therapy of cervical dystonia with BoNT/A seems to be safe and yields good stable results even after 5 years of treatment.

Key words Cervical dystonia · Torticollis · Botulinum toxin · Prospective follow-up study · Antibodies

Introduction

More than 10 years after publication of the initial study by Tsui and coworkers [31] localized injections of botulinum neurotoxin A (BoNT/A) are today considered the treatment of choice in patients suffering from cervical dystonia (CD), also called spasmodic torticollis, the most common form of focal dystonias [27]. A number of controlled double-blind studies have demonstrated de novo BoNT/A injections to be safe and effective in patients with CD [2, 10, 15, 25, 32], and results have been confirmed in open trials with follow-up period of up to 2 years [1, 4, 16].

Although spontaneous remissions occur in up to 10% of cases during the natural course of CD [14] and have also been reported in patients treated with BoNT/A, most patients are confronted with continuing treatment over many years if not life-long. This makes the question of long-term development of symptoms and possible side effects under continuing treatment increasingly important.

However, reports are still sparse on the long-term treatment of the most common form of focal dystonias, namely CD [18]. Most studies to date have concentrated on one of the most important problems in long-term treatment, i.e., the frequency of formation of neutralizing antibodies (Ab) to BoNT/A [9, 17, 34]. Beyond the problem of Ab formation, little is known about other aspects of long-term treatment, such as long-term efficacy, safety, and drop-out rate as a measure of the patient's satisfaction with the treatment.

The present study analyzed data from 303 CD patients treated at our center since 1989 who received at least six local injections of BoNT/A as continuous treatment for at least 2 years. The results of this study have been published previously in abstract form [20]. The findings show clearly that treating CD with BoNT/A injections remains effective even after 16 or more injections. Furthermore, there was no evidence of increased frequency of adverse events (AE), nor were there unexpected or previously unknown reactions to the drug.

Patients and methods

A total of 616 patients suffering from idiopathic CD were treated at our center between October 1988 and December 1995 with local injections of BoNT/A (Dysport, Speywood, U.K.) after having given written informed consent to the treatment. With approval of the local Medical Ethics Committee, standardized forms have been used since 1988 to document basic patient data (date of birth, sex, age at onset of symptoms, duration of symptoms, kinds of symptoms, medical and family history). Patient data and injection follow-up findings were collected in a commercial spreadsheet program (date of injection, total dose and doses by muscle, duration and quality of effect of the previous treatment, occurrence, duration, severity of AE). Additional information was gathered when necessary from the patients' medical records or the patients' primary care physician after obtaining informed consent from the patient.

Most of patients (91.5%) were admitted to hospital for the initial evaluation and treatment. Serum levels of copper and ceruloplasmin, the exclusion of acanthocytosis, and copper excretion in 24-h urine were measured. Further diagnostic procedures such as electroencephalography, computed tomography, or magnetic resonance imaging scan of the brain were performed to exclude a symptomatic form of CD. Prior to the injection a four-channel needle electromyography (EMG) and in some case video documentation were carried out. Written or oral witnessed informed consent was obtained.

Exclusion criteria and subgroups

From the total of 616 datasets on patients suffering from idiopathic CD who presented at our clinic we excluded incomplete datasets (n = 62) and those on patients who initially or intermittently received injections at other clinics (n = 42). Since this study focuses specifically on long-term effects of the treatment, patients receiving fewer than six injections (n = 209) were also excluded. This left a total of 303 completely documented datasets on patients suffering from idiopathic CD. It should be noted that there were no statistically significant differences between this group and the excluded group (n = 313) regarding mean age at onset, mean age at first injection, type of CD, gender ratio, mean BoNT/A dose per treatment session or percentage of AE.

From the total cohort of 616 CD patients 162 did not present for a return visit for more than 12 months and were thus considered as "drop-outs". A semistandardized telephone interview was carried out to ascertain the individual reasons for the dropping out. In seven cases neither the patient nor his or her relatives could be located. All of the remaining 155 patients or, if the patient had died in the meantime, close relatives were interviewed, and it was possible to establish the reason for discontinuing.

Group description

The 303 patients received a total number of 3088 injections, i.e., an average of 10.2 per patient (range: 6–21). The mean age of onset of dystonic symptoms in this group was 41 years (range: 17–69); the female:male-ratio was 1.2:1. The mean duration of symptoms before the first treatment with BoNT/A was 6.5 years (range: 2 months–35 years), and the average time of follow-up after initial presentation was 3.2 years (range: 1.3–5.9 years).

From their histories, 6% of the patients had temporarily suffered from symptoms typical of CD prior to the onset of the current symptoms (relapsing/remitting course). None of these patients reported more than two remissions before CD appeared as a chronic disease with constant symptoms for more than 1 year. Most remissions occurred within the first 18 months after manifestation and lasted up to 11 years. No remissions were reported when the symptoms had persisted for more than 3 years. The most common type of CD in this group was pure rotational CD (torticollis; 40%), followed by a combination of rotational and tilt (laterocollis) component (29%) and pure laterocollis (10%). Pure ante- or retrocollis were the rarest forms, with a total frequency of 8%. In the remaining cases (13%) complex forms of CD were present.

In 19% of the patients the family history was positive for other movement disorders, the most frequent being focal dystonia and tremor. For further details see [23].

Toxin preparation and injection technique

The BoNT/A preparation marketed in the United Kingdom (Dysport) was used in this survey, and the concentration remained the same throughout. The contents of two vials of Dysport, containing a total of 1000 mouse units (MU; i.e., 25 ng toxin-hemagglutinin complex or 4 ng pure toxin), were dissolved in 2.5 ml sterile normal saline solution, yielding a concentration of 400 MU (i.e., 1.6 ng pure toxin) per milliliter. The muscles to be used for the injection were selected by visual analysis of head deviation and identification of dystonic muscles as indicated by hypertrophy, visible and palpable stiffening of muscles, and four-channel polymyographic EMG findings. EMG was performed stereotypically prior to the first injection; at subsequent injections EMG was repeated only if effects were insufficient, or if head posture had changed since previous injections. Injections were carried out with a 2.0-ml syringe and a 27-gauge hypodermic needle guided by anatomical landmarks and palpation of the neck musculature. The sternocleidomastoid muscle (SCM) received injections at one or two sites in the cranial third of the muscle belly, the splenius capitis muscle at three to six separate sites depending on muscle mass and neck diameter, and the trapezius and lateral neck muscles (scalenii or levator scapulae) generally at two or three sites.

Table 1 summarizes the most frequently injected muscles (sternocleidomastoid, splenius, trapezius, and levator scapulae) and the average doses per muscle and session. The clear preponderance of right-sided sternocleidomastoid and left-sided splenius injections reflects the slightly higher prevalence of leftward rotation than rightward rotation in rotational torticollis in this group.

Table 1 Frequently injected muscles and average doses per muscle (*SCM* sternocleidomastoid muscle, *SPL* splenius capitis, *TRA* trapezius muscle, *LEV* levator scapulae)

	SCM		SPL		TRAV		LEV	
	Right	Left	Right	Left	Right	Left	Right	Left
Number of injections	1542	1173	1628	1944	191	202	119	209
Average dose per session	255.4	246.4	423.4	445.5	227.9	242.5	152.8	156.3
Standard deviation of dosage	130.9	120.1	169.0	163.2	128.3	156.9	58.9	61.9

Note the higher number of right-sided SCM and the left-sides SPL injections, indicating the higher prevalence of leftward rotational torticollis

Scoring

The severity of symptoms was rated by the treating physician (R.B.), according to a modified Tsui et al. score [32]. The score was obtained in the following way: Head tremor and shoulder elevation were assessed with the patient sitting erect in a chair with armrests but without support for the head or the upper half of the trunk deviation of normal head position. Deviation from normal head position was rated with 1–3 points, respectively, for head deviation of 0–15°, 15–30°, and over 30° for the dominant component of the CD (either rotational, tilt or yaw) only. This value was multiplied by a factor 1 when the head deviation occurred only intermittently, or by a factor 2 when the head deviation was constant. Head tremor was rated with 0 (absent), 1 (slightly), 2 (moderate), or 3 (severe) additional points with the head in the position in which the patient felt most comfortable. An additional 0–3 points were assigned to the degree of involuntary shoulder elevation. Three points were assigned only when the shoulder made contact with the ipsilateral ear. Finally, the patient's complaint of pain in the neck/shoulder area was scored with 0–3 points for absent, slight or occasional, moderate or severe pain, respectively. Three points were given only if the patient reported a history of frequent prescription of pain killers of oral muscle relaxants. The highest possible total score was 15 points.

The rating was always performed when the patient returned for the next injection, and the scores therefore reflect residual rather than peak effect. A subjective rating scale (percentage reduction in overall symptoms) was used to determine the amount of the peak effect. The time interval between injections was fixed in advance by the physician and in general ranged between 12 and 14 weeks. In very few cases injections were repeated at shorter intervals, sometimes within than 6 weeks ("booster injections"). Severity of AE was rated on a subjective scale ranging from mild (1) to severe (3). For dysphagia, a severity score of 1 was given when the patient noted some difficulty in swallowing but was not bothered by it. A score of 3 was given when the patient had to change the diet, suffered from choking events, or complained of undesired weight loss of more than 4 kg.

Secondary nonresponse

The following clinical criteria were used to define secondary nonresponders: (a) Patients received at least two successful injections (i.e., score improvement of more than 3 points and/or atrophy of an injected muscle) and/or complained of typical BoNT/A related AE. (b) In two subsequent treatment sessions there was no subjective effect or a worsening of the score of more than 2 points and no typical therapy-related AE. These patients were tested for serum Ab using either a mouse bioassay [12], a test injection in the extensor digitorum brevis muscle (EDB test) [19], or a phrenic nerve-hemidiaphragm preparation [11].

Results

Efficacy

Patients reported the onset of benefit an average of 7.5 days (range: 1–42 days) following the injection. According to the patients' subjective impression, the peak effect occurred on average 16 days after injection (range: 7–35 days). The total duration of the effect was found to be 11 ± 2.4 weeks, again, based on the patients' subjective impression, and did not change significantly over time.

Figure 1 shows that treatment decreased the severity of symptoms from 10 points (group median) to 4 points prior to the 15th injection. Improvement was generally most pronounced after the first injection (group median: 6 points) and remained fairly constant after the sixth injection. Over the first six injections the score reduction was highly significant ($P > 0.0001$; Friedman's two-way analysis of variance) and posttests showed significant differences in

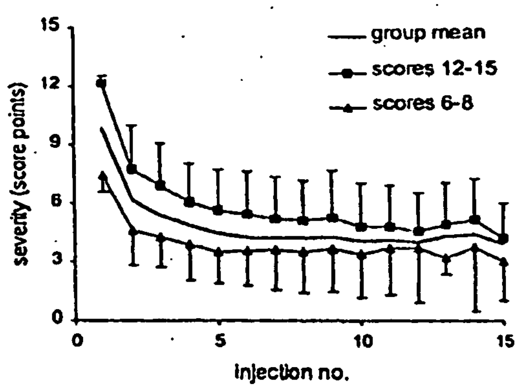


Fig. 1 Development of disease severity (score points) during the course of treatment. *Black squares*, mean scores +1 SD of the subgroup of patients with severe cases of CD (initial scores 12–15); *triangles*, mean scores (−1 SD) of the subgroup of patients with mild CD (initial scores 6–8); *thick black line*, mean scores of the total group. Note that the scores of the initially severely affected patients show a trend to remain higher than those of mildly affected patients

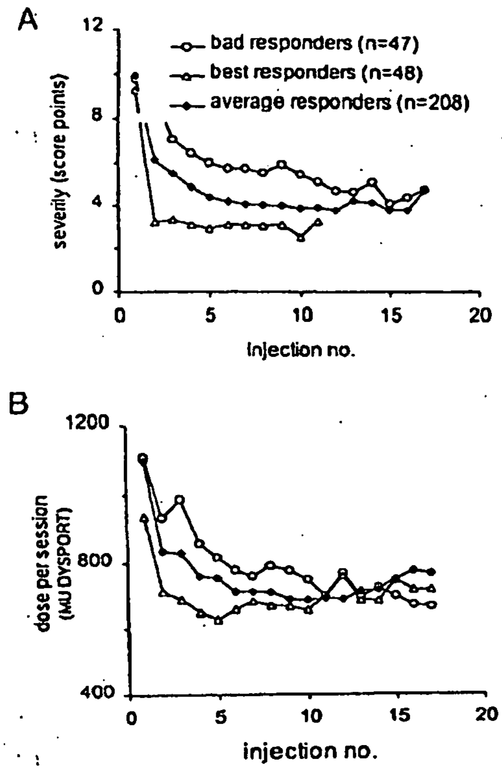


Fig. 2 A, B Development of disease severity (A) and doses per session (B) during the course of treatment. The patients were divided into subgroups by their response to the first injection, for details see text. A All three groups had the same initial score; however, the "poor responders" (*open circles*) continued to have higher scores throughout the course of treatment than the "average" (*black diamonds*) or "good responders" (*open triangles*). B Doses used per session (in mouse units, MU) declined considerably during the course of treatment in all three groups (down-titration). There were no significant differences between the group means of doses, although evidently the "poor responders" were continuously treated with higher doses

scores obtained before the second ($P < 0.0001$) and sixth injections ($P < 0.0001$) compared to the initial score (Wilcoxon's signed-rank test for matched pairs).

Patients generally reported the peak effect to be approx. 30–50% better than the baseline effect. It can therefore be assumed that with a baseline score of 4 the patient was almost asymptomatic around the time of peak effect. Figure 1 also demonstrates that the relative improvement was similar for all patients irrespective of their disease severity. Patients with a higher initial score (range: 12–15) generally continued to have higher baseline scores (difference: 2 points) than patients with lower initial scores (range 6–8).

As was noted above, the greatest relief in symptoms was generally seen following the first injection. The mean score decreased 3.7 ± 1.9 points. As illustrated in Fig. 2, however, 51 patients responded less immediately to the treatment ("poor responders"), i.e., the decrease in score following the first injection was 2 points or less of the group mean, while a group of 49 patients ("good responders") responded better than average (score reduction ≥ 6 points) to the first injection. After six and ten injections the poor responders continued to have higher severity scores than the average or good responders, despite insignificant differences in age at onset of symptoms, disease duration, male:female ratio, or injection dose.

It is important to note that this improvement did not appear to be related to the complexity of CD. No significant difference in efficacy was found between a group of patients with "classical" pure rotational torticollis (n = 124) and a group of complex CD patients (n = 38) showing postural abnormalities in all three directions. This is demonstrated in Fig. 3, showing the mean scores before the first, sixth, and tenth injections; results from the group with pure rotational torticollis are displayed on the left and those of the "complex" group on the right. Wilcoxon's matched pairs test showed a highly significant difference between scores prior to the first and sixth injections for both the rotational ($Z = -5.37$; $P < 0.0001$) and the complex ($Z = -9.542$; $P <$ 0.0001) group. Differences in score values between the two groups were not significant (Mann-Whitney U test).

Dosage

The average total dose per treatment session was 778 ± 253 MU. The initial dose was highly variable and on average (1072 ± 373 MU) also the highest dose used (down titration). During the course of treatment the average total dose was reduced significantly ($P < 0.001$, repeated analysis of variance) and stabilized after approximately six injections at approx. 720 MU.

One factor contributing to the variability of the doses used was the difference in the severity of symptoms. Figure 2 presents the average total doses per treatment session between the three subgroups of good, average, and poor responders. The figure demonstrates that doses were related to the severity of symptoms; doses decreased rather rapidly among good responders but remained high among poor responders until the 10th injection.

Adverse events

AE that may have been related to the BoNT/A injection were reported in 685 of 3088 treatment sessions (22%). Most treatments were accompanied by no complication (it should be noted that data on AE were missing in 6.9% of the records). On the other hand, only 77 of the 303 pa

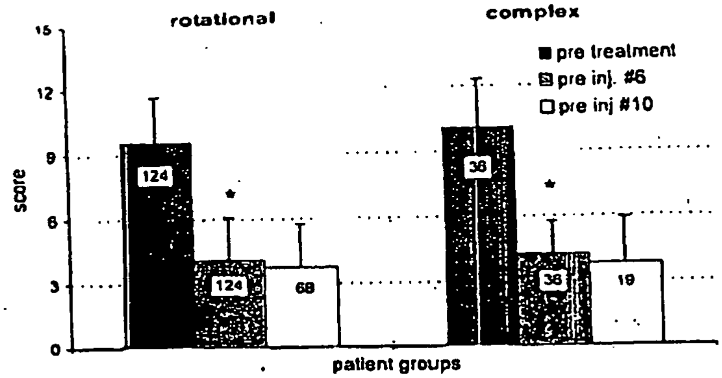


Fig. 3 Comparison of improvement (mean scores) between patients with pure rotational CD (n = 124) and those of complex forms of CD (n = 36). *Black bar*, before first injection; *dark gray bar*, before sixth injection; *light gray bar*, before tenth injection. *Asterisks*; $P < 0.0001$, reduction in score values (Wilcoxon's test for matched pairs); *flags*, +1 SD. The number of observations is given in each bar

tients (25%) experienced no AE of any kind during the course of treatment. The most frequent AE was dysphagia, which occurred on average 9.7 days (range 4–22) following the injection, lasted on average 3.5 weeks (range: 1–8 weeks), and was generally of only low (34%) or moderate (53%) severity. In only 26 patients (4%) was dysphagia so severe that a temporary change in diet was necessary; undesired weight loss of more than 4 kg was noted by the patients. No patient required a nasogastric tube or hospitalization. Other frequently cited AE included neck muscle weakness, hoarseness, and a dry mouth (see Table 2).

There was no evidence of a direct relationship between the occurrence of AE and the total dose injected in a given session. Figure 4 presents the relative frequency of AE as a function of the total dose per session. Over a wide dose range (200–1200 MU) the relative frequency of AE did not differ significantly, remaining at approx. 20%; an increase was seen only with total doses of more than 1200 MU.

Table 2 Spectrum of AE possibly or probably related to the treatment in the order of their frequency as absolute numbers and in percentage of injections followed by AE (n = 685)

	n	%
Dysphagia	528	77.1
Neck muscle weakness	118	17.2
Dry mouth	68	9.9
Neck pain	32	4.7
Voice changes	29	4.2
General weakness	16	2.3
Respiratory difficulties	13	1.9
Facial muscle weakness	8	1.2
Visual disturbance	6	0.9
Vertigo, nausea	4	0.6
Other	14	2.0
Total	836	122.0

The relative number of AE amounts to more than 100%, because some patients named more than one AE

Although the probability of experiencing AE decreased with increasing number of treatment sessions, a significant number of patients experienced AE in the later course of the treatment. As shown in Fig. 5, almost 50% of patients who experienced AE after the second injection had had none after the first injection, and even after ten injections almost 25% of AE occurred for the first time.

The mean total dose tended to be slightly higher in injections followed by AE (802.1 ± 282.5 MU) than in those without AE (750.5 ± 209.6 MU), this difference was statistically nonsignificant.

Injection of the SCM showed a barely significant association with subsequent dysphagia. Dysphagia followed

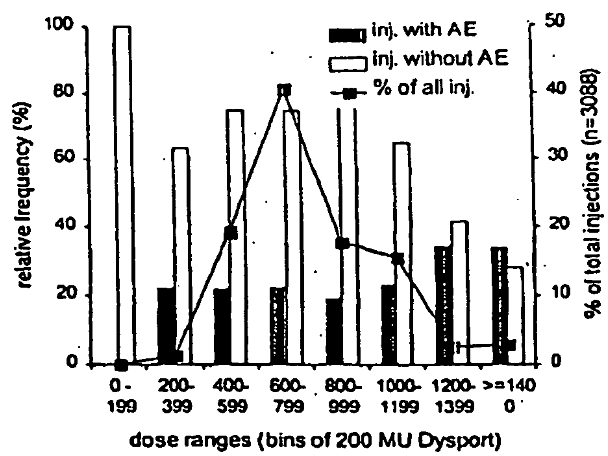
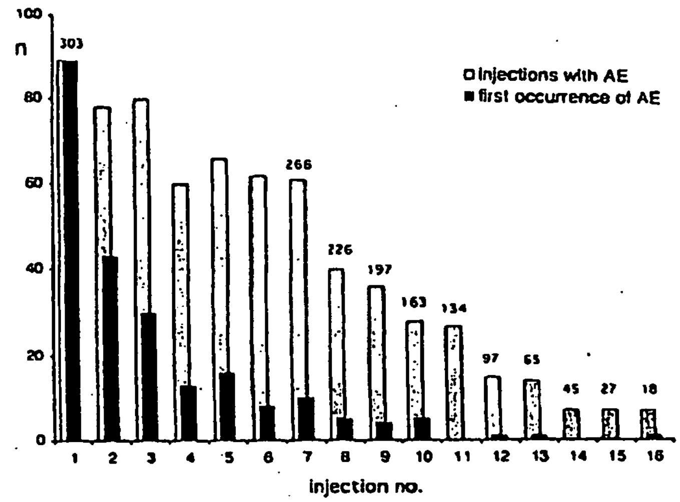


Fig. 4 Frequency of the occurrence of AE in relation to the total dose per session. Relative frequency of injections without AE (*open bars*) and with AE (*gray bars*) in percentage of the total number of injections (*y-axis*) in relation to the total dose per session in bins of 200 MU Dysport; *y-axis*, relative frequency of injections in the given dose ranges, demonstrating that in approx. 90% of injections doses ranged from 200–1200 MU with a peak in the 600–800 MU range

Fig. 5 Occurrence of AE through the course of treatment. *Light gray bars*, number of injections after which AE were reported by the patient against number of treatment (*x-axis*); *dark gray bars*, number of cases in which this was the first time in the course of treatment that the patient reported an AE at all. The figure illustrates the decreasing frequency of occurrence of AE during the course of treatment. *Numbers above bars*, number of patients, which is constant (n = 303) up to the sixth injection



13.5% (55/428) of treatment sessions in which the SCM was not injected at all but in 17% (456/2605) of sessions in which SCM was injected unilaterally (P = 0.043) and in 25% (14/55) of those in which SCM was injected bilaterally (P = 0.049). The difference between unilateral and bilateral injections, however, was statistically not significant (P = 0.231, Fisher's exact test).

Although many AE have been reported in the past, no new type of AE was encountered with long-term treatment in this series. The rate of AE related to autonomic function, such as dizziness, was almost negligible, and no increase in such reports was observed with lengthening duration of therapy.

Treatment discontinuation

Of the total cohort of 616 CD patients treated at our center the 155 who were lost to follow-up but were available for a telephone interview cited a total of 173 reasons for discontinuation. As Table 3 shows, a large group of those patients had not actually discontinued BoNT/A therapy but had merely switched to other treatment centers located closer to their homes. The corrected overall drop-out rate was thus 20% (126/616 patients). The two next most common reasons for discontinuing treatment were "unsatisfactory treatment result" (20%) and, in contrast, "satisfactory" or "complete and persistent" symptom relief (16%). Twenty-six patients even reported that their symptoms had been "completely abolished"; on a subjective rating scale the others reported an average symptom relief of 80% which had persisted for 17 months on average at the time of the interview.

Another 17% of drop-outs reported AE as their principal reason for discontinuation, the most frequently named of which was, again, dysphagia, which in two cases had been so severe as to require nasogastric tube feeding for 8 and 17 days, respectively. It is important to note the time at which patients gave up the treatment; half of them discontinued therapy after the first, second, or third injection. Again, approximately half of these early drop-outs reported an unsatisfactory effect or AE the reason for discontinuation. As indicated above (see Fig. 2), in individual patients the beneficial effect of the treatment may be delayed, and it may be therefore assumed that the actual "unsatisfactory effect" rate could be lower.

Table 3 Reasons for treatment discontinuation

	n	% of total
Changes of treatment center	36	21.1
Unsatisfactory or no effect	33	19.3
Adverse events	27	15.8
Satisfactory or complete relief	26	15.2
Other	19	11.1
Secondary nonresponse	17	9.9
Discontinued by physician	8	4.7
Unknown	7	4.1
Total	173	101.2

Using a standardized telephone interview, 155 patients were asked to give their individual reasons for not returning to the center. Medial contraindications were Coumarin therapy (4), pregnancy (3), neuromuscular disease (1). Other reasons included 9 patients who had died (4 of cancer, 2 of cardiovascular disease, 3 of unknown case, apparently unrelated to therapy). None of the patients had died within 3 months following an injection. The category "unknown" included 7 patients who could not be located for interview

Table 4 Characteristics of secondary nonresponders and responders. Booster injections were defined as injections within 6 weeks following the previous injection (medians, range)

	Secondary nonresponders (n = 17)		Responders (n = 303)	P[a]
	Ab⁺ (n = 9)	Ab⁻ (n = 8)		
Age at onset of symptoms (years)	30 (13–51)	39 (28–55)	41 (8–50)	0.007[b]
Duration of symptoms				
Before treatment (months)	38 (19–121)	47 (12–192)	58 (2–426)	n.d.
Before nonresponse (months)	36 (20–53)	40 (29–72)	–	n.d.
MU per session	875 (400–1750)	820 (400–2000)	750 (150–2250)	0.0001[b]
Cumulative dose (MU)	9000 (6455–12.405)	9675 (8475–18.050)	7430 (2700–22.475)	0.0822[b]
Interval (days)	91 (11–271)	91 (3–273)	105 (1–874)	0.0001[b]
No. of injections	11 (7–14)	14 (9–22)	10.2 ± 3.2	n.d.
Booster	4/96 injections (4.2%)	7/117 injections (5.9%)	41/3089 injections (1.3%)	0.049[c,b]

Nonparametric statistical comparisons (two-tailed Mann-Whitney U tests; χ^2 only for the booster injections) were carried out between the Ab⁺ group and the responding group

[a] For comparisons between Ab⁺ secondary nonresponding and responding patients

[b] Mann-Whitney U test (two-tailed)

[c] Fisher's exact test for 2 × 2 tables

Secondary nonresponse

Among the 162 patients who discontinued therapy, 17 reported having lost their initially beneficial effect. (For the clinical definition of secondary nonresponse see "Patients and methods") Table 4 summarizes the major features of this group. At least one of the tests performed detected neutralizing serum Ab in 9 of the 17 patients who clinically fulfilled the criteria for secondary nonresponse. Since secondary nonresponse was never seen before the sixth injection, the reference group should be restricted to patients with six or more injections (303 still on therapy plus 54 drop-outs), resulting in a minimum Ab frequency of 2.5% (9/357). As Table 4 indicates, the Ab⁺ secondary nonresponders and the responding controls differed significantly in three aspects: the dose per session was higher (P < 0.0001), treatment intervals were shorter (P < 0.0001), and the number of booster sessions (defined as a reinjection within 6 weeks of the previous treatment) was higher (P = 0.049) in the former. The difference in doses per session was significant (P = 0.0002) even when the last two injections, for which the protocol demanded an increase in dose, were omitted from the analysis.

Remarkably, the only patient characteristic which showed a significant difference between groups was age: Ab⁺ patients were younger at the onset of dystonic symptoms than those in the responder group (P = 0.007).

Discussion

Although BoNT/A therapy of CD was first reported in 1985 [31], this is the first prospective follow-up study encompassing up to 16 injections over approx. 4 years and in a reasonable number of patients.

This study clearly shows that BoNT/A is a highly effective treatment for CD even over a prolonged period of time. This treatment had earlier been expected to produce finite results most patients, with a lasting improvement; however, it has become clear in recent years that therapy will be continuous in most patients. Using a modified Tsui score to document efficacy, the present study found a highly significant average score reduction from 10 points on initial presentation to 4 points after 15 injections. Earlier short-term studies using the original score as proposed by Tsui et al. in 1985 have reported less pronounced decreases in score, ranging from 20% to 47% [4, 6, 30, 31]. One of the criticisms of the original Tsui score was that it rates solely head deviation and tremor, while pain, which is a major contributor to disability in most CD patients was rated separately [29] in order to avoid mixing objective and subjective items in a single score. For this study and also for clinical purposes, the original score was modified in that only the dominant direction of head deviation was rated, the relative weight of tremor was reduced, and a pain score was added. These modifications may have contributed to the highly significant change in scores found in this study, although Poewe at al. [28] have also reported improvements in a similar range using the original score. We feel that the modified score used here adequately reflects the overall development of impairment from CD as determined from subjective global patient ratings.

The data presented here clearly show that most patients undergo dramatic improvements after the first few injections, and that scores stabilize between the fourth and eighth injection. Considering that 32 patients who discon-

tinued therapy gave no or insufficient treatment benefit as the reason for their discontinuation, the overall success rate in the present study can be estimated at around 90%, similar to that reported previously [14, 24, 28].

It should also be noted that our results indicate that BoNT treatment is equally effective in "simple" rotational torticollis and in more complex forms of CD. This finding is not at all obvious since it could be expected that complex forms of CD involve a greater number of and possibly also deeper lying muscles. Because no prospective comparative study had previously addressed the question of efficacy in relation to the various types of CD, drug regulatory authorities in a number of countries (including Germany) have approved of the use of BoNT injections only for rational torticollis. This study provides the first evidence that BoNT may be equally effective in more complex forms of CD.

Safety aspects

It should be noted that initial doses were used as a rule in our center (down-titration). This may in part for account the dramatic effects seen, especially after the first injection. However, the AE rate was also quite high following the first injection (27% of injections), and the dose per treatment was significantly reduced in subsequent treatment sessions. AE then occurred less frequently, following some 20% of injections, which is comparable to the rate reported in previous studies using the same toxin preparation with similar average total dose per session [2, 22, 28, 33]. It is of interest that even at the reduced dose, typical toxin-related first-time AE occurred late during the course of treatment, for example, after the 10th or 12th injection, while all previous injections had been uncomplicated. This may be due either to increasing muscle atrophy or to accidental systemic application. This barely significant association between dysphagia and SCM injections suggests that muscle atrophy and the spread of relatively more toxin to neighboring muscles is the most likely explanation for first-time AE such as dysphagia and neck muscle weakness later in treatment. It is important for both the physician and the patient to be aware of the fact that every new reinjection is associated with a certain risk of AE, bearing in mind that in this study 75% of patients experienced AE at least once during the course of their treatment.

There are a number of published reports indicating that, despite all precautions taken by the treating physicians, the toxin can exhibit systemic effects, such as increased muscle fiber jitter in remote muscles and changes in autonomic functions, for example, heart rate variability [8, 21]. However, there is no indication either from previous investigations [18, 26] or from the present study that systemic effects become clinically relevant during long-term exposure to BoNT/A.

Interindividual differences in the response to BoNT injections

An unexpected finding in this study were the interindividual differences in response to treatment, despite the absence of differences in the initial degree of disease severity or complexity of dystonia. The most probable explanation for this finding is that the "wrong" muscles were sometimes chosen for injection. However, this is unlikely to explain the group differences fully since the limited response in patients was seen not only after one injection but over the first few injections, despite adjustment of doses and treatment sites. Associated skeletal or ligamentous changes (contracturelike states) are also an unlikely explanation since no significant reduction in score would be expected except a reduction in pain. A better explanation would be the involvement of remote muscles (e.g., deeper neck muscles) which were not routinely the site of injection. Finally, individual differences in treatment response may have been caused by differences in individual's biological response. Given the complicated mechanism of action requiring acceptor-mediated active endocytosis, the biological prerequisites (e.g., acceptor density on the nerve terminal) cannot be expected to be the same in all individuals.

As a practical consequence of our finding we suggest that both the treating physician and the patient be aware of the profound differences in the effect of the treatment. This could reduce the likelihood of disappointment and hence the rate of early drop-outs due to and "false" primary nonresponders. The results presented here indicate that the true quality of treatment cannot reliably be judged before the sixth to eighth injections, especially in patients who initially do not respond well.

Secondary nonresponse

Secondary nonresponse is one of the major problems in long-term treatment of CD with BoNT/A because it entails discontinuing treatment, depriving the patient of the most successful therapy available. It is by now well accepted that one of the major causes for the loss of treatment response is the formation of neutralizing serum Ab to BoNT/A. The toxin is known to be immunogenic; both laboratory personnel handling *Clostridium botulinum* and military personnel have been successfully immunized with a polyvalent toxoid after three subcutaneous injections at baseline, after 2 and 12 weeks, and a booster injection after 1 year [13].

Determining the true frequency of secondary nonresponse in a given cohort is impeded by two confounding factors: the clinical definition of secondary nonresponse and technical problems with currently available Ab tests. The mouse bioassay, which determines the presence of neutralizing Ab by measuring mouse LD_{50} [12, 13], has to

date been considered the gold standard, despite its lack in sensitivity [3].

In 1993 Zuber et al. [34] were the first to report three secondary nonresponders, defined by clinical criteria in a group of 96 patients with Dysport. In the following year Greene et al. [9] reported Ab detection in 4.35% of their CD patients ($n = 559$) treated with Botox (Allergan, USA) between 1984 and 1992. Ab frequency was 10.5% when an appropriate subgroup was analyzed. Duane et al. [5] found 15 secondary nonresponders in a group of 98 patients (15.3% suffering from CD) and neutralizing serum Ab in 10 of these patients, using a mouse bioassay. Finally, Jankovic and Schwartz [17] reported "lack of adequate response" in 54 of 1322 patients (4.1%), of whom 20 tested positive for Ab in a mouse bioassay.

In the present study the rate of secondary nonresponse with neutralizing Ab amounted to 2.5%, which is comparable to the rate reported by Zuber et al. (3%) using the same toxin preparation (Dysport). As the most pessimistic estimate, one could speculate that lack of test sensitivity led to Ab remaining undetected in all of the eight Ab− nonresponders; however, even then the Ab frequency in this study would be 4.8% (17/357). Having used at least two of three tests available in each of our secondary nonresponders, however, we feel confident that this would actually overestimate the true Ab frequency.

The present study confirms that patients at risk of developing neutralizing Ab are those with high doses administered at relatively short intervals, which is in good agreement with previous studies on this issue. It should be stressed that no data are presently available to indicate whether one of these two factors is more important. It is also noteworthy that in regard to the above risk factors Ab^+ and Ab^- patients did not differ significantly. This could be due either to an unknown proportion of false-negative secondary nonresponders or to the patients' demands and the physician's attempts to improve efficacy by preferring higher doses. Possible causes of secondary nonresponse in the absence of serum Ab have been addressed in previous studies [17, 19]. Considering the criteria for defining secondary nonresponse in the present study, which included an increase in injected dose, it appears unlikely that inappropriate injection sites or doses are major contributing factors. Unfortunately, there was no data available to determine whether some or all of these patients underwent a change in the pattern of dystonic neck muscle, which has been suggested to be a main cause of secondary nonresponse by Gelb at al. [7]. Whether other biological changes in the nerve terminal occur that could lead to resistance to the toxin other than that which is Ab mediated (e.g., down-regulation of the acceptor protein) is still speculative.

The significantly younger age at onset of symptoms in the Ab^+ group than in the other groups has previously been noted also by Jankovic and Schwartz [17], but the relevance of this finding in unclear at present.

At the present time we emphasize the recommendation by Greene and coworkers [9, 10] that CD treatment intervals in CD cases should be longer than 10 (preferably 12 weeks), and that doses as low as possible should be used.

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References

1. Benecke R, Kessler KR (1994) Die Behandlung zervikaler Dystonien mit Botulinum Toxin A. In: Huffmann G, Braune J, Henn KH (eds) Extrapyramidale Bewegungsstörungen. Einhorn, Reinbek, pp 408–417
2. Blackie JD, Lees AJ (1990) Botulinum toxin treatment in spasmodic torticollis. J Neurol Neurosurg Psychiatry 53: 640–643
3. Borodic G, Johnson E, Goodnough M, Schants E (1996) Botulinum toxin therapy, immunologic resistance, and problems with available materials. Neurology 46: 26–29
4. Ceballos-Baumann AO, Konstanzer A, Dengler R, Conrad B (1990) Lokale Injektionen von Botulinum-Toxin bei zervikaler Dystonie: Verlaufsbeobachtungen an 45 Patienten. Akt Neurol 17: 139–145
5. Duane D, Clark M, LaPointe LL, Case JL (1995) Botulinum toxin A antibodies in initial and delayed resistance to Botulinum toxin A therapy in cervical dystonia. Mov Disord 10: 394 (A 82)
6. Gelb DJ, Lowenstein DH, Aminoff MJ (1989) Controlled trial of botulinum toxin injections in the treatment of spasmodic torticollis. Neurology 39: 80–84
7. Gelb DJ, Yoshimura DM, Olney RK, Lowenstein DH, Aminoff MJ (1991) Change in pattern of muscle activity following botulinum toxin injections for torticollis. Ann Neurol 29: 370–376
8. Girlanda P, Vita G, Nicolosi C, Milone S, Messina C (1992) Botulinum toxin therapy: distant effects on neuromuscular transmission and autonomic nervous system. J Neurol Neurosurg Psychiatry 55: 844–845
9. Greene P, Fahn S, Diamond B (1994) Development of resistance to botulinum toxin type A in patients with torticollis. Mov Disord 9: 213–217
10. Greene P, Kang U, Fahn S, Brin M, Moskowitz C, Flaster E (1990) Double-blind, placebo-controlled trial of botulinum toxin injections for the treatment of spasmodic torticollis. Neurology 40: 1213–1218
11. Habermann E, Dreyer F, Bigalke H (1980) Tetanus toxin blocks the neuromuscular transmission in vitro like botulinum A toxin. Naunyn Schmiedebergs Arch Pharmacol 311: 33–40
12. Hambleton P, Cohen HE, Palmer BJ, Melling J (1992) Antitoxins and botulinum toxin treatment. BMJ 304: 959–960

13. Hatheway CL, Dang C (1994) Immunogenicity of the neurotoxins of Clostridium botulinum. In: Jankovic J, Hallet M (eds) Therapy with botulinum toxin. Dekker, New York, pp 93–107
14. Jahanshahi M, Marion MH, Marsden CD (1990) Natural history of adult-onset idiopathic torticollis. Arch Neurol 47:548–552
15. Jankovic J, Orman J (1987) Botulinum A toxin for cranial-cervical dystonia: a double-blind, placebo-controlled study. Neurology 37:616–623
16. Jankovic J, Schwartz K (1990) Botulinum toxin injections for cervical dystonia. Neurology 40:277–280
17. Jankovic J, Schwartz K (1995) Response and immunoresistance to botulinum toxin injections. Neurology 45:1743–1745
18. Jankovic J, Schwartz KS (1993) Longitudinal experience with botulinum toxin injections for treatment of blepharospasm and cervical dystonia. Neurology 43:834–836
19. Kessler KR, Benecke R (1997) The EDB test – a clinical test for the detection of antibodies to botulinum toxin type A. Mov Disord 12:95–99
20. Kessler KR, Skutta M, Benecke R (1995) Long-term treatment with botulinum toxin A (BTX A): efficacy, safety, and antibody frequency. Mov Disord 10:385
21. Lange DJ, Rubin M, Greene PE, Kang UJ, Moskowitz CB, Brin MF, Lovelace RE, Fahn S (1991) Distant effects of locally injected botulinum toxin: a double-blind study of single fiber EMG changes. Muscle Nerve 14:672–675
22. Lees AJ, Turjanski N, Rivest J, Whurr R, Lorch M, Brookes G (1992) Treatment of cervical dystonia hand spasms and laryngeal dystonia with botulinum toxin. J Neurol 239:1–4
23. Leube B, Kessler KR, Goecke T, Auburger G, Benecke R (1997) Frequency of familial inheritance among 488 index patients with idiopathic focal dystonia and clinical variability in a large family. Mov Disord 12:1000–1006
24. Lorentz IT, Subramaniam SS, Yiannikas C (1991) Treatment of idiopathic spasmodic torticollis with botulinum toxin A: a double-blind study on twenty-three patients. Mov Disord 6:145–150
25. Moore AP, Blumhardt LD (1991) A double blind trial of botulinum toxin "A" in torticollis, with one year follow up. J Neurol Neurosurg Psychiatry 54:813–816
26. Nebe A, Schelosky L, Wissel J, Ebersbach G, Scholz U, Poewe W (1995) Influence of botulinum toxin type A on the autonomic nervous system in patients with cervical dystonia. Mov Disord 9 [Suppl 1]:45
27. Nutt JG, Muenter MD, Aronson A, Kurland LT, Melton LJd (1988) Epidemiology of focal and generalized dystonia in Rochester, Minnesota. Mov Disord 3:188–194
28. Poewe W, Schelosky L, Kleedorfer B, Heinen F, Wagner M, Deuschl G (1992) Treatment of spasmodic torticollis with local injections of botulinum toxin. One-year follow-up in 37 patients. J Neurol 239:21–25
29. Poewe W, Wissel J (1994) Experience with botulinum toxin in cervical dystonia. In: Jankovic J, Hallet M (eds) Therapy with botulinum toxin. Dekker, New York, pp 267–278
30. Stell R, Thompson PD, Marsden CD (1988) Botulinum toxin in spasmodic torticollis. J Neurol Neurosurg Psychiatry 51:920–923
31. Tsui JK, Eisen A, Mak E, Carruthers J, Scott A, Calne DB (1985) A pilot study on the use of botulinum toxin in spasmodic torticollis. Can J Neurol Sci 12:314–316
32. Tsui JK, Eisen A, Stoessl AJ, Calne S, Calne DB (1986) Double-blind study of botulinum toxin in spasmodic torticollis. Lancet 2:245–247
33. Wissel J, Poewe W (1992) Dystonia – a clinical, neuropathological and therapeutic review. J Neural Transm Suppl 38:91–104
34. Zuber M, Sebald M, Bathien N, de Recondo J, Rondot P (1993) Botulinum antibodies in dystonic patients treated with type A botulinum toxin: frequency and significance. Neurology 43:1715–1718

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Botulinum A Toxin Therapy: Neutralizing and Nonneutralizing Antibodies—Therapeutic Consequences

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Although muscle-relaxant doses of botulinum A toxin (BoNT/A) are generally lower than doses stimulating the immune system, specific antibodies are raised in a substantial number of patients. As a rule, this necessitates the termination of treatment. Therefore, a reliable determination of specific anti-BoNT/A antibodies is helpful and we introduced, for this purpose, a novel *in vitro* toxin-neutralizing assay based on a nerve-muscle preparation. We measured the antibody titers in four groups of subjects: Group 1 comprised 75 randomly selected patients of a total of 295 who responded to treatment with Dysport in our local clinic. Five patients, in group 2, were nonresponders. Group 3 consisted of 32 untreated volunteers and group 4 of 8 subjects immunized with a toxoid more than 10 years ago. Two of the responders had marginal titers of neutralizing antibodies, while they were present in all nonresponders. The sera of all responders were also tested for nonneutralizing antibodies by ELISA. Their occurrence, however, was of no consequence to the therapeutic success. The blood samples of volunteers were free from specific antibodies, whereas antibodies persisted in the immunized subjects for longer than a decade. Patients from various clinics who had been treated unsuccessfully with the toxin—14 patients had received BOTOX, 7 had been treated with Dysport, and 7 with both products—all had neutralizing antibodies. Whether there was an antibody response depended on the amount of toxin administered. We believe, however, the effective toxin dose can be reduced by so much as to make antibody production highly improbable. © 1997 Academic Press

INTRODUCTION

Botulinum ~~A toxin is the drug~~ of choice to improve several forms of ~~focal dystonia~~ (13, 26). Its scope of therapy, however, has widened immensely during the recent years. To date, not only disorders characterized by abnormal contractions of striated muscles, like tremor (14), tics (21), facial wrinkles (9, 20), and spasticity (5, 27), are treated with it, but also vegetative disturbances that manifest themselves in certain gastrointestinal ailments (18, 19, 23) and gustatory sweating (25). As a consequence the number of reports of acquired failure of therapy is growing (2, 3, 10). The main reason why nerve endings lost their sensitivity to the toxin was attributed to the production of neutralizing anti-botulinum toxin antibodies (8, 15, 17, 29). ~~But~~ since ~~the toxin in use is not a purified protein,~~ there may also be antibodies ~~directed against the accompanying proteins~~ (e.g., hemagglutinins) which ~~account for~~ at least ~~70% of the total mass of the complex formed by~~ the toxin ~~and concomitant~~ proteins. Antibodies appeared particularly in patients who had received higher doses than those necessary for the treatment of blepharospasm and facial spasm (6, 16, 29) and the existence of a dose relationship seems probable. Moreover, if antibodies are formed, it is unclear how long their production will be sustained in patients who fail to respond to treatment. Also, botulinum toxins share amino acid sequences with tetanus toxin (22), and polyclonal antibodies have been described that neutralize both toxins by recognizing a common epitope (1). Thus, despite the differing immunogenicities of clostridial neurotoxins, cross-reactivity may exist and patients immunized against tetanus toxoid might also be immune to botulinum A neurotoxin. In the present work we have characterized various types of antibodies (neutralizing and nonneutralizing). We have determined dose–immune response relationships and, judging from the persistence of antibodies in immunized subjects, have estimated how much time may have to elapse until a secondary nonresponder can reacquire his sensitivity to the toxin.

MATERIALS AND METHODS

Materials

Botulinum A toxin (BoNT/A) complex (LD_{50} 35pg mouse) was from Dr. E. Schantz, Madison, Wisconsin. This toxin was used for the determination of the calibration curve and the antibody titers of sera. The active constituent consists of two molecules of neurotoxin linked to hemagglutinin and a nonagglutinating protein. The complex, also termed the 19 S complex,

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has a molecular weight of 900 kDa. The neurotoxin accounts for 30% of the total mass (12). Our own hemagglutinin-free neurotoxin was purified according to DasGupta and Sathyamorthy (4). Its LD_{50} was 10 pg/mouse.

Tetanus toxin (TeNT) was from U. Weller, Mainz, Germany. Bovine serum albumin (protease-free) was from SERVA (Heidelberg, Germany). All buffers were prepared with analytical-grade chemicals from Merck (Darmstadt, Germany). Specific anti-botulinum A toxin $F(ab')_2$ (equine Fermo serum, 750 U/ml) was from Behringwerke (Marburg, Germany). It served as a standard for the construction of the calibration curve. Anti-human IgG from goat and anti-horse IgG from rabbit, both conjugated with peroxidase, were from Dako (Denmark). Krebs–Ringer solution was composed of 118 m*M* NaCl, 4.75 m*M* KCl, 2.54 m*M* $CaCl_2$, 1.19 m*M* KH_2PO_4, 1.2 m*M* $MgSO_4$, 1.2 m*M* $NaHCO_3$, 11 m*M* glucose, and 0.1% bovine serum albumin. The toxins were diluted in Krebs–Ringer solution to a final concentration of 1 ng/ml for the purified neurotoxin and 2 ng/ml for the complex. For ELISA, wells were coated with toxin solutions free of protective albumin.

Experimental Procedures

The left phrenic nerve–hemidiaphragm was excised from male or female NMRI mice (18–22 g), placed in an organ bath containing 3.5 ml of Krebs–Ringer solution, and stimulated via the N. phrenicus (28). Indirectly stimulated muscles (1 Hz, 1 ms, 1 V) maintained an undiminished contractile response (twitch) over 4 h. In each experiment, the preparation was first allowed to equilibrate for 15 min under control conditions. Then, the incubation medium was exchanged for the toxin-containing serum. Before mixing the toxin with the undiluted sera, sera were dialyzed against the Krebs–Ringer solution. The mixtures were then incubated for 60 min at 37 ℃. Appropriate toxin concentrations were used to allow the contraction amplitude, in the absence of antibodies, to be reduced by half in approximately 60 min. After application of the mixture, the amplitude remained unchanged for some time (Fig. 1a), then decreased more or less slowly, depending on the antibody titer in the serum. The time required to decrease the amplitude by 50% (paralysis time, always less than 3 h) served to construct the calibration curve using the anti-botulism serum from Behring (750 U/ml) as a standard. When paralysis time exceeded 180 min in the presence of a patient's serum, the serum was diluted to allow paralysis to develop within that time. Taking the dilution factor into account, the antibody titer was determined with the help of the calibration curve.

ELISA tests were performed using 96-well plates

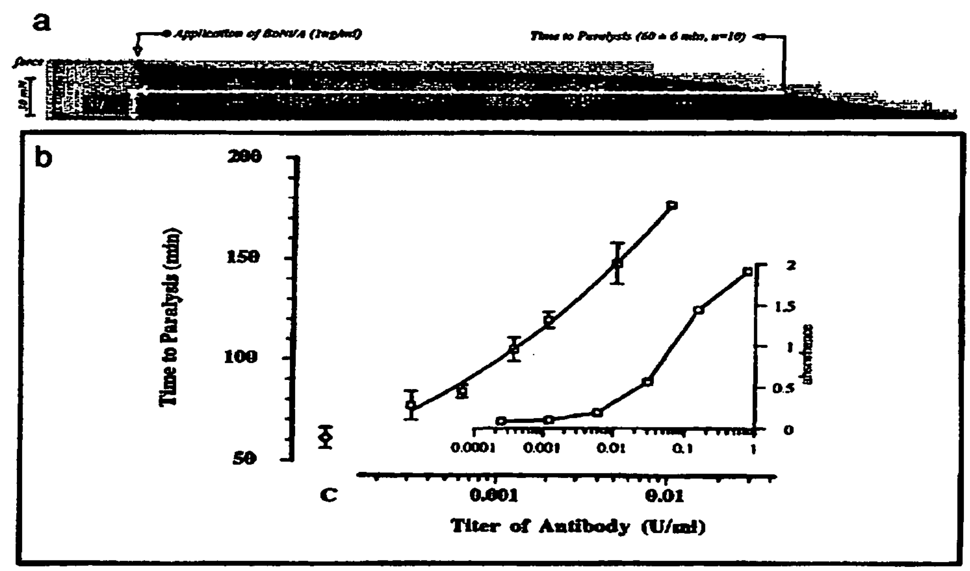

FIG. 1. Development of paralysis and antibody titer. (a) A mouse diaphragm was continuously stimulated via the phrenic nerve at a frequency of 1 Hz. After equilibration the muscle was exposed to 1 ng/ml of botulinum toxin. The arrows indicate when the toxin was applied and when the twitch was reduced by 50% of its initial value, respectively. Paralysis time is defined as the time elapsed till the contraction amplitude has been halved. (b) Calibration curves of a standard anti-botulinum A toxin serum: Antibody titer plotted against time to paralysis in an *in vitro* experiment using the mouse hemidiaphragm ($n = 3 \pm$ SD). The diamond symbol C (for control) represents the paralysis time of diaphragms exposed to a mixture of antibody-free human sera and 2 ng/ml of toxin complex ($n = 35 \pm$ SD). Inset, Antibody titer plotted against absorbance in an ELISA test ($n = 2$).

coated overnight with 100 ng/ml of the antigen (TeNT, BoNT/A, and BoNT/A–hemagglutinin complex, respectively) at 4 °C in sodium phosphate buffer (20 m*M*) containing NaCl (150 m*M*) (PBS). After being washed with the buffer, the plates were incubated with bovine serum albumin (10 mg/ml) in PBS to block nonspecific binding to the plates. The anti-botulism serum from Behring (horse, 750 U/ml) was diluted in PBS and used for the determination of the titer/response curve (Fig. 1b, inset). These dilutions and the human sera, diluted 1:3 and 1:10 with the same buffer, respectively, were applied to the plates and incubated for 8 h. The wells were washed again and incubated with the anti-horse and anti-human IgG peroxidase conjugates, respectively. Upon washing, the plates were developed with benzidine, and the absorbance was measured in an EIA reader at 450 nm.

RESULTS

Determination of Anti-botulinum Toxin Antibodies Using the Phrenic Nerve–Hemidiaphragm Preparation and ELISA

Botulinum toxin type A complex (2 ng/ml) and hemagglutinin-free neurotoxin (1 ng/ml), added to dialyzed sera of antibody-free controls, both paralyzed the hemidiaphragm and reduced its contraction amplitude by 50% within 61 ± 5 ($n = 32$) and 60 ± 6 min ($n = 10$), respectively (Fig. 1a). The time to paralysis was prolonged depending on the titer of specific antibodies which was adjusted with a standard botulism antitoxin from horse (Fig. 1b). The detection limit of toxin-neutralizing antibodies was approximately 0.0003 U/ml. Using the ELISA technique, the detection limit of neutralizing and nonneutralizing anti-botulinum toxin antibodies was approximately 0.01 U/ml. This means that the ELISA is approximately 300 times less sensitive than the muscle preparation and, moreover, cannot discriminate between toxin-neutralizing and -nonneutralizing antibodies.

Quantification of Botulinum Toxins of Various Origins

One hundred units of Dysport equals 2.5 ng botulinum toxin complex (11), while 100 U of BOTOX resides in 25 ng of the protein complex (24). One molecule of the complex (M_r of 900,000) contains two molecules of neurotoxin (M_r of 150,000) (12). On this basis concentration–response curves were calculated (Fig. 2). Dysport was as potent as our own purified neurotoxin. The concentration–response curve of BOTOX was shifted to the right by a factor of 10; i.e., taking account of molarity, BOTOX had to be applied in a 10-fold higher concentration than Dysport to attain an equipotent response.

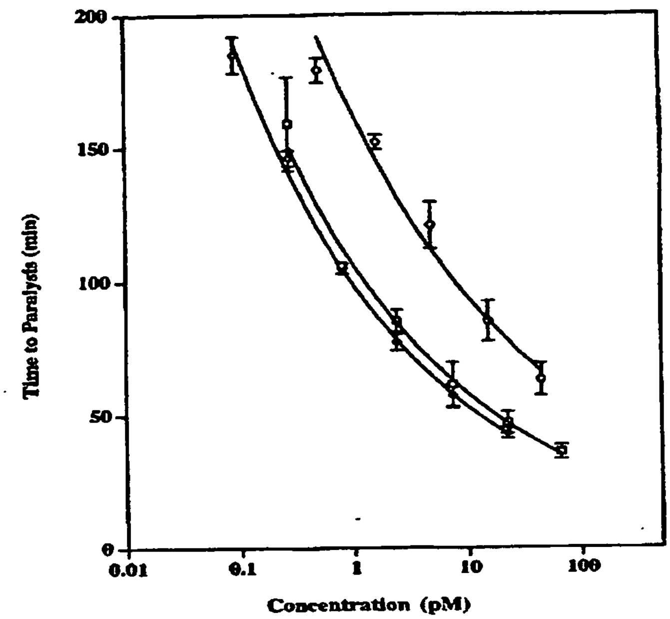

FIG. 2. Concentration–response curves for purified botulinum A neurotoxin, BOTOX, and Dysport. Mouse hemidiaphragms were exposed to varying amounts of three different botulinum toxin preparations: purified neurotoxin type A (□), Dysport (◇), and BOTOX (○). Concentrations in pmol/liter (abscissa) were plotted against time up to the point when the contraction amplitude had decreased to 50% of its initial value (ordinate). Each point is the mean of three experiments. Variance is expressed as ±SD. Where no deviation bar is shown, it is concealed by the symbol.

Antibody Titers in Sera of Dysport-Treated Patients from Hannover, Volunteer Blood Donors, and BoNT-Immunized Subjects

Within a period of 15 months, 300 patients suffering from various forms of dystonia consulted the Neurological Clinic of Hannover Medical School. Females accounted for 58% and males for 42%. The average age was 50.8 years, the mean duration of treatment was 5.5 years, and the mean interval between injections was 3.2 months. Fifty percent of these patients suffered from torticollis spasmodicus, 35% from facial dystonias, 10% from generalized torsion dystonia, and 5% from spasticity. All were treated with Dysport, the doses varying according to their requirements (Table 1). Among these patients were 4 secondary nonresponders and 1 primary nonresponder. Patients were considered nonresponders when they showed neither improvement nor muscle weakness or atrophy after at least two successive treatments. The primary nonresponder, a 42-year-old man, had been suffering from torticollis spasmodicus for 11 years. Then in 1994 he had four unsuccessful treatments with Dysport and two with BOTOX. The intervals between the injections were always 2 months. The sera of 75 responders and all

nonresponders were tested for botulinum toxin complex-neutralizing antibodies, using the nerve–muscle preparation. The sera of those patients, in particular, who received high-dose treatment (>600 U) were selected for the test. In contrast to the sera of nonresponders, all of which contained antibodies of various titers, detectable titers of specific antibodies were found in the sera of only two responders (Fig. 3; Table 1). The mean dose of Dysport administered to secondary nonresponders was 20 ± 5 ng (823 ± 194 U) (Table 2). The lowest dose that induced antibodies was 15.5 ng (620 U). Thus, of the group of patients receiving doses higher than 15 ng of Dysport (n = 40), approximately 10% produced neutralizing antibodies. Serum samples from volunteer blood donors (n = 32) were free of botulinum toxin complex-neutralizing antibodies (Fig. 3). Each serum, however, contained anti-tetanus toxin antibodies (>0.1 U/ml), indicating a successful immunization with tetanus toxoid (see Discussion). Subjects immunized with botulinum toxoid (n = 8) had neutralizing titers of anti-botulinum toxin A antibodies although they had not received booster injections for 10 years (Fig. 3).

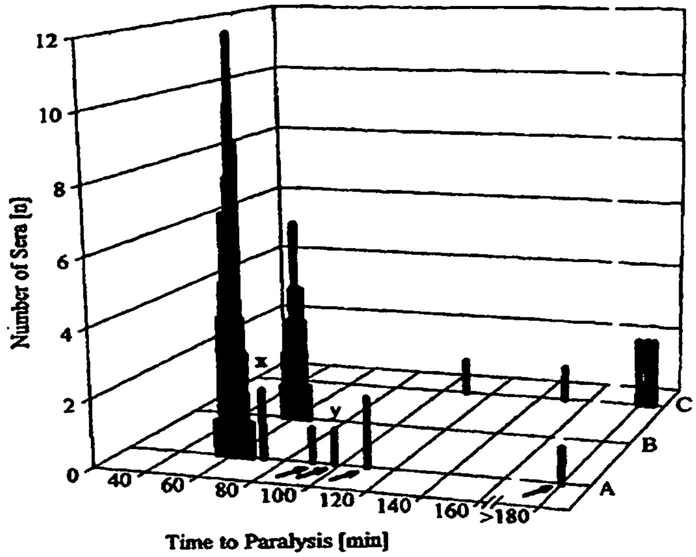

FIG. 3. Paralysis times in three groups of subjects. Mouse hemidiaphragms were exposed to 2 ng/ml of botulinum A toxin complex mixed (A) with the dialyzed sera of patients treated with Dysport, (B) with dialyzed sera of volunteer blood donors, and (C) with dialyzed sera of botulinum toxoid-immunized subjects. Columns show numbers of sera (ordinate) that paralyze the diaphragm after the times indicated on the abscissa. The column marked "x" represents the sera of two patients which contained traces of antibodies, the column marked "y" represents the serum of a patient who did not respond to the toxin in the first place, and the arrows mark the columns that represent the sera of nonresponders.

Antibody Titers in Sera of Patients Treated Outside of Hannover

We received the sera of 44 suspected nonresponders from various clinics all over Germany. Detectable titers of botulinum toxin complex-neutralizing antibodies were found in the sera of 29 patients. All of the remaining, antibody-negative, patients (n = 15) responded to subsequent injections of BoNT/A, as confirmed by the attending physicians. Of the antibody-positive patients, 14 had been treated with BOTOX. Twelve of them had received mean doses of 48 ± 14 ng (192 ± 56 U) each, while we had no information about the remaining 2 patients. Seven patients had received mean doses of 20 ± 2 ng (800 ± 92 U) of Dysport (Table 2). The latter dose comes close to the mean dose administered to antibody-positive patients from Hannover. Seven patients had received injections of both products, and no information at all was available on the treatment of 1 patient (Table 2). Sex and age of patients, diagnosis, and treatment are shown in Table 3.

TABLE 1

Data for Dysport-Treated Patients from Hannover

	Low dose	Medium dose	High dose
Range (ng)	1.0–7.4	7.5–15.0	>15.0
Range (U)	40–299	300–600	>600
Patients treated (n)	140	120	40
Sera tested (n)	20	26	30
Nonresponders (n)	0	1	4
Time to paralysis (min)			
Responders (means ± SD)	61 ± 3	62 ± 3	61 ± 3
One "primary" nonresponder		98	
Secondary nonresponders			90, 110, 112, >180
Positive ELISA with toxin complex (nonneutralizing antibodies)			
n	8	11	14
in percentage of sera tested	40	42	47

Note. Patients treated with Dysport were divided into three groups according to the doses applied. Secondary nonresponders received doses of more than 600 U, i.e., 15 ng. Nonneutralizing antibodies (positive ELISA with toxin complex) were found more frequently in sera of patients treated with Dysport in the upper dose range.

Botulinum Toxin-Neutralizing vs -Nonneutralizing Antibodies

The muscle preparation was protected from the action of the toxin complex in the presence of sera containing neutralizing antibodies which, however, may have been directed against any of the various constituents of the complex. We therefore tested the same sera (from 32 patients) on muscle preparations exposed to purified neurotoxin. This way it should be possible to distinguish neurotoxin-specific antibodies from those directed against some other part of the complex. The latter would be unable to prolong time to paralysis in this experiment. The results showed that neutralizing

TABLE 2

Antibody Titers in Sera of Dysport and BOTOX-Treated Nonresponders

Dysport						BOTOX					
				Mean dose						Mean dose	
Patient	Titer (U/ml)	ELISA (neurotoxin)	Range (U)	(U)	(ng)	Patient	Titer (U/ml)	ELISA (neurotoxin)	Range (U)	(U)	(ng)
1	0.09	+	970–1360	1130	28.0	12	>0.05	+	100–300	170	42.5
2	0.002	−	200–1040	700	17.5	13	0.09	+	50–240	150	37.5
3	0.002	−	400–760	620	15.5	14	0.5	+	75–240	155	39.0
4	0.005	−	800–900	840	21	15	0.03	+	120–350	200	50.0
1–4 mean				822.5	20.5	16	0.01	−	250–300	280	70.0
(±SD)				(194.2)	(4.7)	17	0.01	+	120–350	250	62.5
5	0.04	−	500–980	700	17.5	18	0.003	−	100–350	250	62.5
6	0.6	+	500–1500	1000	25.0	19	0.01	−	80–120	100	25.0
7	0.01	+	480–1040	720	18.0	20	0.004	−	85–150	115	29.0
8	0.02	−	600–1000	825	21.0	21	0.018	−	200–360	240	60.0
9	0.07	−	400–1000	810	20.0	22	0.014	−	100–300	200	50.0
10	0.32	+	700–900	800	20.0	23	0.16	+	100–250	200	50.0
11	0.6	+	500–1000	750	18.7	24	0.001	−	?	?	?
						25	0.014	+	?	?	?
5–11 mean				800.7	20.0	12–25 mean				192.5	48.2
(±SD)				(92.3)	(2.3)	(±SD)				(56.3)	(14.0)
1–11 mean				808.6	20.2						
(±SD)				(138.7)	(3.4)						
26	0.004	−	760–1200	1000	25				10–100	60	15
27	0.04	−	450–960	700	17.5				100	100	25
28	0.01	+	800–960	880	22.0				30–120	60	15
29	0.014	+	680–760	720	18.0				170–230	200	50.0
30	0.4	+	450–1000	760	19.0				50–260	215	53.8
31	0.002	−	540–1000	750	18.7				160–260	200	50.0
32	0.12	+	320–900	400	10.0				60–300	100	25.0
33	0.02	+	?	?	?				?	?	?

Note. The titers of nonresponders are juxtaposed with the mean doses administered. Values 1 to 4 are from patients treated with Dysport in our local clinic. Mean values were calculated separately for the patients from Hannover (1–4), from outside Hannover (5–11 and 12–25 for Dysport and BOTOX, respectively), and for both (1–11, Dysport-treated). The values at the bottom (26–32) represent patients treated with both products while no information at all was available for one patient (33). Question marks indicate a lack of information about the doses of BOTOX and Dysport. The ELISA was performed with hemaglutinin-free botulinum A neurotoxin (see Materials and Methods).

antibodies were exclusively directed against the neurotoxic constituent of the complex. The titer measured had the same height as the titer against the toxin complex (results not shown). In addition, through the use of the ELISA technique, all sera containing neutralizing antibodies ($n = 32$) were tested for neurotoxin-specific antibodies (Table 2) and, conversely, antibody-negative sera ($n = 72$) for nonneutralizing antibodies (Table 1) aimed at some other part of the complex. Neurotoxin-specific antibodies could be detected in only 43% of the antibody-positive sera which contained titers of 0.01 U/ml or higher (Table 2). In sera of responders antibodies against the complex were present that did not neutralize the neurotoxin (Table 1).

DISCUSSION

It is common practice to measure by units both the titer of specific antibodies and the activity of botulinum toxin preparations destined for use in therapy. To avoid confusion, we have calculated the doses of both commercial products, i.e., BOTOX and Dysport, on the basis of protein content. The conversion appears justified because, in contrast to the paralytic action which depends solely on the proteolytic activity of the neurotoxins, the immune response is almost exclusively determined by the protein contents of the preparations. The potency loss of BOTOX which, in relation to protein content, amounted to 90% (Fig. 2), probably resulted from the formation of toxoid. Toxoid formation had previously been attributed to lyophilization of botulinum toxin in a solution containing a high concentration of sodium chloride (7). The 10-fold higher protein concentration per unit of BOTOX may compensate for the loss of potency but, on that order, might adversely affect the immune system.

A very reliable *in vitro* neutralization test has been introduced to detect and quantify antibodies raised in patients during treatment with botulinum A toxin. Toxicity tests in mice are terminated by the paralysis of

TABLE 3

Data from Patients Who Did Not Respond to Either Dysport or BOTOX

				Treatment	
Patient	Sex	Age (years)	Diagnosis	Duration (years)	Interval (months)
1	M	37	GTD	4	3.7
2	M	33	GTD	3	2.3
3	F	80	SPA	2	2.0
4	M	61	TC	3	3.0
5	F	38	TC	2	2.7
6	M	31	TC	5	2.7
7	F	55	GTD	2	1.2
8	F	56	TC	3	2.8
9	M	45	TC	4	2.4
10	M	45	TC	3	2.3
11	M	50	TC	3	3.3
12	M	58	TC	3	4.5
13	F	46	TC	2	3.0
14	M	44	TC	3	4.5
15	F	23	TC	2	4.8
16	F	53	TD	2	3.4
17	M	23	GTD	5	3.8
18	F	69	TC	3	5.1
19	F	61	OMD	4	3.7
20	F	70	TC	4	4.0
21	M	52	?	2	3.4
22	F	59	TC	2	4.8
23	F	26	TC	4	5.3
24	F	?	?	?	?
25	F	41	?	?	?
26	M	55	TC	7	7.0
27	M	68	TC	4	2.8
28	F	60	TC	2	2.2
29	M	73	TC	3	3.0
30	M	35	TC	5	5.0
31	F	62	TC	3	3.3
32	F	36	TC	4	5.3
33	?	?	?	?	?

Note. The numbers in the first column correspond to the numbers in Table 2. Numbers 1–4 represent the patients from our local ambulance and 5–33 those from outside Hannover. The data refer to 1996. GTD, generalized torsion dystonia; SPA, spasticity; TC, torticollis spasmodicus; OMD, oromandibular dystonia.

the respiratory muscles. Testing in the isolated respiratory muscle is a truncated version of the animal test and has the advantage of avoiding complex pharmacokinetics. This test is approximately 300 times more sensitive than the ELISA technique and can detect amounts of neutralizing antibodies so small as to be of no consequence for therapy. Thus, antibodies near the detection limit (0.0003 U/ml) were found in the sera of two patients who still responded to treatment (see Fig. 3, values marked with "x"). Patients with titers higher than 0.001 U/ml, however, were resistant to therapeutic doses of botulinum A toxin. Neutralizing antibodies, as the cause of therapeutic failure, were also identified with the help of the mouse *in vivo* toxin neutralization assay (17). Knowledge of the antibody status can be helpful in showing the way to proceed in cases of unsatisfactory responses to therapy. Thus, it was found that neutralizing antibodies were not present in the 15 putative secondary nonresponders. Whatever the causes of initial failure may have been, the resumption of therapy was a success.

When exposed to purified botulinum A neurotoxin or botulinum toxin complex, respectively, the nerve-muscle preparation allowed discrimination between the sera that contained antibodies directed against the neurotoxin and those with antibodies against the stabilizing proteins like the hemagglutinins which do not contribute to paralysis. Anti-hemagglutinin antibodies were detected with the ELISA technique and were found in patients who still responded to the toxin. In contrast to patients with neutralizing antibodies, those testing positive for nonneutralizing antibodies will continue to benefit from the toxin.

One patient did not respond to botulinum toxin from the very beginning. In searching for an explanation for this failure, it had to be considered that he might have had cross-reactive anti-tetanus antibodies, because both toxins share a 35% homology in amino acid sequence (22) and, at least under experimental conditions, neutralizing antibodies against a common epitope have been found (1). Therefore, we tested the sera of a group of volunteer blood donors, successfully immunized against tetanus, for their capacity to neutralize botulinum toxin. None of the sera, however, showed anti-botulinum reactivity, so that cross-reactive antibodies are improbable to account for refractoriness. Moreover, it is unlikely that the non-responder may have acquired immunity through an undiagnosed botulism. Possibly, he may have reacted to the small initial doses of toxin with an unusually rapid production of antibodies.

The initiation of the production of neutralizing antibodies is more probable when large amounts of protein (neurotoxin) are applied, as could be shown for patients receiving Dysport. Of all local patients treated with high doses (>15 ng) 10% became resistant to the toxin by raising antibodies. A dose correlation was also seen for antibodies formed against the concomitant proteins in the complex (Table 1). Corresponding data of patients treated with BOTOX are not available yet because we had sera only from putative secondary nonresponders at our disposal. However, it is reasonable to surmise that, also in the case of BOTOX, the production of antibodies is linked to the amount of protein applied.

Since detectable antibodies were raised only when the mean dose (in nanograms) of BOTOX was more than twice as high (48 ng) as the mean dose of Dysport (20.2 ng), it has to be considered that the ratio between the protein contents of the two products may be 1:5 rather than 1:10. In any case, based on the number of units, the mean dose of Dysport (808 U) to give rise to

antibodies is approximately four times as high as the mean dose of BOTOX (192 U) (see also Table 2). This advantage of Dysport over BOTOX is forfeited by the current practice of administering doses three to five times higher than is necessary to achieve the therapeutic goal (28). Needless overdosing can do considerable harm to the patients because they may have to discontinue the treatment if antibodies are formed. These may persist for more than a decade, as tests on immunized subjects have shown. Therefore, a patient who develops immunity would possibly be denied treatment for the rest of his life.

Based on the present experience a second generation of botulinum toxin preparations should answer the following criteria: (i) The preparations should be devoid of toxoid. This will keep the risk of sensibilizing the immune system to a minimum. (ii) The toxin should also be purified from concomitant proteins. This will reduce the load of foreign substances that might lead to untoward reactions. [A case of rash linked to Dysport has recently been communicated to the authors (Christian Figge, unpublished).] Also, the concomitant proteins might serve as adjuvants that stimulate the production of anti-neurotoxin antibodies. (iii) Package sizes should be adequate to allow maximum exploitation of biological activity, as has been discussed recently elsewhere (28).

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REFERENCES

1. Bartels, F., H. Bergel, H. Bigalke, J. Frevert, J. Halpern, and J. Middlebrook. 1994. Specific antibodies against the Zn^{2+}-binding domain of clostridial neurotoxins restore exocytosis in chromaffin cells treated with tetanus and botulinum A neurotoxin. *J. Biol. Chem.* **269:** 8122–8127.
2. Borodic, G., E. Johnson, M. Goodnough, and E. J. Schantz. 1996. Botulinum toxin therapy, immunologic resistance, and problems with available materials. *Neurology* **46:** 26–29.
3. Critchley, E. M. R. 1995. Non-responders. *Mov. Disord.* **10:** 392.
4. DasGupta, B. R., and V. Sathyamorthy. 1984. Purification and amino acid composition of type A botulinum neurotoxin. *Toxicon* **22:** 415–424.
5. Dengler, R., U. Neyer, K. Wohlfarth, U. Bettig, and H. H. Janzik. 1992. Local botulinum toxin in the treatment of spastic drop foot. *J. Neurol.* **239:** 375–378.
6. Gonnering, R. S. 1988. Negative antibody response to long-term treatment of facial spasm with botulinum toxin. *Am. J. Ophthalmol.* **105:** 313–315.
7. Goodnough, C. M., and E. A. Johnson. 1992. Stabilization of botulinum toxin type A during lyophilization. *Appl. Environ. Microbiol.* **58:** 3426–3428.
8. Greene, P., S. Fahn, and B. Diamond. 1994. Development of resistance to botulinum toxin type A in patients with torticollis. *Mov. Disord.* **9:** 213–217.
9. Guyuron, B., and S. W. Huddelston. 1994. Aesthetic indications for botulinum toxin injection. *Plast. Reconstr. Surg.* **5:** 913–918.
10. Hambleton, P. 1994. Immunogenicity aspects of botulinum toxin. Pages 16–19 in *Botulinum Toxin: Challenges and Developments.* LMB Public Relation Survey.
11. Hambleton, P., and A. M. Pickett. 1994. Potency equivalence of botulinum toxin preparations. *J. R. Soc. Med.* **87:** 719.
12. Inoue, K., Y. Fujinaga, T. Watanabe, T. Ohyama, K. Moriishi, H. Sunagawa, K. Inoue, and K. Oguma. 1995. Molecular composition of progenitor toxins produced by C. botulinum types A and D. *Jpn. J. Med. Sci. Biol.* **48:** 263–264.
13. Jankovic, J., and M. F. Brin. 1991. Therapeutic uses of botulinum toxin. *N. Engl. J. Med.* **324:** 1186–1194.
14. Jankovic, J., and K. Schwartz. 1991. Botulinum toxin treatment of tremors. *Neurology* **41:** 1185–1188.
15. Jankovic, J., and K. Schwartz. 1991. Clinical correlates of response to botulinum toxin injections. *Arch. Neurol.* **48:** 1253–1256.
16. Jankovic, J., and K. Schwartz. 1993. Longitudinal experience with botulinum toxin injections for treatment of blepharospasm and cervical dystonia. *Neurology* **43:** 834–836.
17. Jankovic, J., and K. Schwartz. 1995. Response and immunoresistance to botulinum toxin. *Neurology* **45:** 1743–1746.
18. Jost, W. H. 1995. Botulinum toxin in the treatment of anismus and spastic pelvic floor syndrome. *Mov. Dis.* **10:** 404.
19. Jost, W. H., and K. Schimrigk. 1995. Botulinum toxin in the therapy of anal fissure. *Lancet* **344:** 1127–1128.
20. Keen, M., A. Blitzer, J. E. Aviv, W. Binder, J. Prystowsky, H. Smith, and M. Brin. 1994. Botulinum toxin A for hyperkinetic facial lines: Results of a double-blind, placebo-controlled study. *Plast. Reconstr. Surg.* **94:** 94–99.
21. Krack, P., U. Teschendorf, and W. Dorndorf. 1995. Modification of a facial tic with botulinum toxin. *Mov. Disord.* **10:** 401.
22. Niemann, H. 1991. Molecular biology of Clostridial neurotoxins. Pages 303–348 in J. E. Alouf and J. H. Freer, Eds., *Sourcebook of Bacterial Protein Toxins.* Academic Press, San Diego.
23. Pasricha, P. J., W. J. Ravich, T. R. Hendrix, S. Sostre, B. Jones, and A. N. Kalloo. 1995. Intrasphincteric botulinum toxin for the treatment of achalasia. *N. Engl. J. Med.* **332:** 774–778.
24. Schantz, E. J., and E. A. Johnson. 1990. Dose standardisation of botulinum toxin. *Lancet* **335:** 421.
25. Schulze-Bonhage, A., M. Schröder, and A. Ferbert. 1996. Botulinum toxin in the therapy of gustatory sweating. *J. Neurol.* **243:** 143–146.
26. Scott, A. B., B. G. Kennedy, and H. A. Stubbs. 1985. Botulinum A toxin injection as a treatment for blepharospasm. *Arch. Ophthalmol.* **103:** 347–350.
27. Snow, B. J., J. K. Tsui, M. H. Bhatt, M. Varelas, S. H. Hashimoto, and D. B. Calne. 1990. Treatment of spasticity with botulinum toxin: A double blind study. *Ann. Neurol.* **28:** 512–515.
28. Wohlfarth, K., H. Göschel, J. Frevert, R. Dengler, and H. Bigalke. 1997. Botulinum A toxins: Units vs. units. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **355:** 335–340.
29. Zuber, M., M. Sebald, N. Bathien, J. de Recondo, and P. Rondot. 1993. Botulinum antibodies in dystonic patients treated with type A botulinum toxin: Frequency and significance. *Neurology* **43:** 1715–1718.